EFFECTS OF PREVIOUS TREE HARVESTING ON PRODUCTIVITY AND PHOTOSYNTHETIC PIGMENTS OF MOSSES IN A BOREAL BLACK SPRUCE FOREST

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Abstract

The harvesting of forests across Canada is known to affect the carbon fluxes of these ecosystems over large scales, but little is known about the potential range of longer term effects on the bryophytes which cover the forest floor. This study aimed to investigate seasonal productivity of common moss species (*Hylocomium splendens*, *Ptilium crista-castrensis*, *Pleurozium schreberi*, and *Sphagnum subnitens*) from black spruce boreal forest sites which had previously been clearcut in Western Newfoundland, Canada, in comparison to the same moss species in adjacent intact forests. Tests focused mainly on the photosynthetic rates and photosynthetic pigment concentrations of the species, and found contrasting results. Feathermosses tested in the post-harvest areas had greater photosynthetic rates in the more open habitats, but the decreased rates of growth coupled with the higher vapour pressure deficits measured in these areas suggests that they were often under moisture stress and were unable to capitalize on their light environment. *Sphagnum* was able to better retain water in these open areas, and had a smaller treatment effect. The light responses of all three feathermosses were such that saturating light levels were greater in the more open post-harvest blocks, and concentrations of photosynthetic pigments decreased as light was no longer a limited resource. In contrast, *Sphagnum* was able to increase maximum photosynthetic rates in the post-harvest blocks, and fewer effects of treatment were found when measuring photosynthetic pigments, again suggesting that *Sphagnum* shoots were more capable of mitigating water loss and associated effects. All the test species were found to have naturally increased shoot densities in the post-

harvest blocks, presumably an attempt to mitigate the negative effects of a more challenging micro-environment. Overall, mosses were found to be capable of maintaining a substantial ground cover within the post-harvest areas, but did display a range of changes to characteristics and traits which could potentially alter their proportional contribution to the carbon fluxes in a harvested area.

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Co-authorship statement

I conducted this research independently but under the co-supervision of Dr. Jianghua Wu and Dr. Xinbiao Zhu. I was responsible for substantial components of the project development and design in collaboration with my committee members. I completed the field research associated with this project with the assistance of Mr. Gordon Butt and Mrs. Rebekah Stone and the summer student Mrs. Miranda Wiseman who worked under my supervisor.

I collected, entered, and analyzed all data based on consultation with Dr. Jianghua Wu, Dr. Xinbiao Zhu, and Dr. Raymond Thomas. In addition, I interpreted all of the results and wrote the manuscripts that constitute the chapters of this thesis. I have revised the manuscripts based on the advice and comments of my supervisor, and supervisory committee.

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Chapter 1: Introduction and Overview

1.1 Introduction

Within Canada the boreal ecosystem is important both economically and biologically, with more than half a million hectares harvested annually for timber and wood products (Natural Resources Canada, 2014). Up to 80% of the boreal forest area harvested annually is done by clearcutting, a process by which all merchantable trees are removed (Youngblood and Titus, 1996). Clearcutting can impact local environmental conditions both in the short and longer term; most often noted are increases in ground-level temperature and light levels that reach the ground (Bergeron *et al.* 2009). These potential abiotic environment changes can impose stress on plant-life, reducing growth and development or changing magnitudes and rates of carbon (C) fluxes within these systems (Arsenault *et al.* 2012; Lichtenthaler, 1996).

The C fluxes within an environment are dominated by photosynthesis of plant matter, which removes carbon dioxide (CO_2) from the atmosphere, and by respiration, which emits CO_2 (Bonan, 1991). Undisturbed boreal forests are generally considered net C sinks, meaning that the flux due to photosynthesis is greater on an annual basis and C is stored within the system (Bonan, 1991). Given the importance of harvesting and concerns over future climate changes, a better understanding of C cycling through boreal forests is crucial so that more accurate C budgets can be estimated.

The main effects of tree harvesting are increases in air temperature, soil temperature, and light levels at ground level (Arsenault *et al.* 2012). The greater openness of the area once the tree canopy is removed also allows for more wind to pass through,

and with higher light levels and air temperatures, this can lead to increased evaporation rates (Skre *et al.* 1983). Understory vegetation is highly influenced by the overstory dynamics within the boreal forest, and changes to the canopy can alter the presence and ground cover of many forest floor species (Chipman and Johnson, 2002). The removal of overstory vegetation can allow smaller shrubs to colonize and their canopies often have less spaces between leaves and branches through which light can reach the ground, thereby decreasing the light available to short ground cover species and impacting the future regeneration of overstory vegetation (Hart and Chen, 2006).

Picea mariana (Mill.) B.S.O. (black spruce) forests occur throughout the boreal region which spans Canada (Bona *et al.* 2013; Rowe, 1972). Boreal black spruce forests are characterized by forest floors with a relatively high percentage or essentially complete ground cover of bryophytes (Bergeron *et al.* 2009; Okland and Okland, 1996), though these ground floor bryophytes are often overlooked in traditional tree harvest studies efforts are now being made to include them in research studies (Nelson and Halpern, 2005; Arsenault et al. 2012; Hart and Chen, 2006).

The bryophyte group encompasses mosses, liverworts, and hornworts, with mosses as the dominant taxa (Lindo and Gonzalez, 2010; Wood, 2007). It is generally thought that mosses have conserved much of their basic structure over their evolution, but species have adapted such that an extensive range of habitats are colonized (Hübers and Kerp, 2012; Turetsky *et al.* 2012). Mosses have relatively recently been recognized as good indicator species for local environmental change (Arsenault *et al.* 2012), due mostly to their lack of common plant structural components and poikilohydric nature (Botting and Fredeen, 2006).

The effects of harvesting specifically on mosses is important, as it has been estimated that up to 80% of the boreal C pool stored in soils can be attributed to organic inputs from the bryophyte layer on the forest floors (Benscoter and Vitt, 2007), and their net primary productivity can exceed that of the over-story vegetation (Bisbee *et al.* 2001; Goulden and Crill, 1997). Apart from contributing to the C cycles of their environments, bryophytes can also alter and change soil thermal regimes, play a role a nutrient cycling, and affect the local hydrology (Bisbee *et al.* 2001, Jonsson *et al.* 2015, Müller *et al.* 2016). Mosses can be particularly susceptible to changes in microclimate conditions as they lack many of the water retention features of vascular plants and are therefore more acutely impacted by changes to the moisture regime (Arsenault *et al.* 2012; Proctor 1990). Generally, mosses decrease their proportional ground cover and nutrient content in areas that have been clearcut (Nelson and Halpern, 2005; Palvianen et al. 2005). However, some studies have found an increase in forest floor moss biomass with increasing harvest intensity when comparing clearcut and partial-cut plots to uncut areas (Lee *et al.* 2002).

Three feathermoss species common to the boreal forest and with more widespread potential in detecting environmental changes are *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) Schimp., and *Ptilium crista-castrensis* (Hedw.) De Not. *P. schreberi* is a pleurocarpous moss and one of the most common ground-cover species in the boreal (Rice *et al.* 2008; Benscoter and Vitt, 2007). Shoot and branch growth occurs both apically and laterally, extending from previous years' growth to a determinate horizontal length, at which point branch growth stops (Tobias and Niinemets, 2010; Rice *et al.* 2008). There is no distinct marker between annual growth segments, and the shoots have a tapered shape at the upper end (Benscoter and Vitt, 2007). *H. splendens* has a

widespread range across the boreal and highly visible markers between annual growth segments (Okland and Okland, 1996). Annual growth occurs typically from a single growth point on the main axis during the spring, and by the fall the new growth has developed branches but will only reach maturity near the end of the following summer (Okland and Okland, 1996). *P. crista-castrensis* grows in a distinct feather shape, with monopodial branching and a shoot developed from a single apical cell (Benscoter and Vitt, 2007; Pederson *et al.* 2001). The branches are all of similar length, except for near the tip where branches are shorter, leading to a feather-like shape (Pederson *et al.* 2001). *Sphagnum* species are commonly found on the ground of black spruce forests in wetter areas with relatively low tree density (Bisbee *et al.* 2001). In areas where *Sphagnum* is present, the plants will often cause an increase in soil moisture due to their high water holding capacity, such that the soil becomes waterlogged and lower in pH (Bates and Farmer, 1992; Bisbee *et al.* 2001).

During and after periods of changing microclimate, moss shoots can alter both physiological and functional traits in order to increase their fitness, most common among these adaptations are changing photosynthetic responses to light levels and altering photosynthetic pigment concentrations (Davey and Rothery, 1996; Hoddinott and Bain, 1979; Lichtenthaler *et al.* 2013). On a larger scale these changes are expressed by altered growth rates, proportional ground cover, and C flux rates over a season (Bansal *et al.* 2012; Bu *et al.* 2011; Gignac, 2001). For all species, the more energy allocated to water storage adaptations, be it a denser mat, more branching, or a greater amount of hyaline cells, the less energy can be used to create photosynthetic cells (Rice *et al.* 2008).

Mosses lack true roots and vascular systems to draw up water from depth, and require external water sources to regulate their water content, given this they require adequate water inputs through precipitation, humidity, or high water tables, or in an area where evaporation is reduced due to lower light levels (Busby *et al.* 1978; Marschall and Proctor, 2004). Many mosses are characterized by the strong relationship between water content and photosynthesis rates, with photosynthetic activity constrained at high and low water contents; photosynthesis of boreal mosses is reported to reach a peak at water contents between 2-6g g^{-1} (Busby and Whitfield, 1978). Mosses are highly dependent on adequate moisture levels for photosynthesis, the amount of time during which shoots can photosynthesize after water input is highly variable among species due to differences in morphology and growth form (Proctor, 1990).

Mosses reach maximum photosynthesis rates at species specific optimum water content, with net photosynthesis decreasing when water content is raised or lowered (Williams and Flanagan, 1996); at low water contents many species can temporarily cease metabolic activity though this does come at the cost of high respiration rates upon rewetting, while at high water contents CO_2 diffusion into the cells can be hampered (Turetsky *et al.* 2012; Proctor 1990). The ability of mosses to resume metabolic activity upon rewetting in an environment is dependent on the severity of desiccation that it endured, with longer events potentially being fatal to shoots upon rewetting (Proctor, 1990). Seasonally, mosses alter their net productivity based largely on the moisture regime of an area; in temperate regions net photosynthesis is often greatest for shoots in the spring and fall when moisture levels are less limiting (Bates *et al.* 2005).

Given the small shoot size and limited water adaptations present in mosses, it follows that they do best in low light environments where evaporation risks are lowest, and forest floor species often reach maximum photosynthesis rates at relatively low light levels (Marschall and Proctor, 2004). In order to mitigate the negative effects of these low light environments, many mosses exhibit light saturation for photosynthesis at comparatively low light levels (Bergeron *et al.* 2009). The response of photosynthesis to changing light levels is most often tested by creating light response curves, testing rates of photosynthesis at many light levels often ranging from 0-1000 μmol $m^{-2}s^{-1}$ (Peek *et al.* 2002; Rice *et al.* 2008). The photosynthetic response curve typically exhibits relatively rapid and linear increases in photosynthesis at low light levels (<100 μmol $m^{-2}s^{-1}$), when photosynthesis is limited by the rate of electron transport (Bubier *et al.* 1999; Farquhar *et al.* 1980). Photosynthesis rates during high light periods is limited by Rubisco capacity and reaches a maximum photosynthesis rate before in levelling off, though in reality at high light levels photosynthesis rates decrease due to photo-inhibition (Farquhar *et al.* 1980; Harley *et al.* 1989). Photo-inhibition occurs at relatively low irradiance levels in many bryophyte species, and occurs as a result of excessive light energy which cannot be dealt with through normal photosynthesis, leading to excessive excitation energy which hampers the process (Deltoro *et al.* 1998). Feathermosses were found to be light saturated at light levels around 200 μmol $m^{-2}s^{-1}$, generally mirroring daytime irradiance levels in natural conditions (Bergeron *et al.* 2009). Moss shoots from higher light environments have been found to have lower rates of maximum quantum efficiency, greater saturating light levels, greater net CO_2 assimilation rates, and greater light requirements to attain a net CO_2 assimilation rate of 0 (Hájek *et al.* 2009; Lichtenthaler, 1996).

Mosses can alter their mat density and shoot size in response to microclimate conditions, though this can come at a physiological cost to individual shoots (Bates, 1988; Okland and Okland, 1996; Pederson *et al.* 2001; Tobias and Niinemets, 2010). A negative relationship between shoot size and stem density has been found for some species of *Sphagnum* (Clymo, 1970), while the relationship for feathermosses is more variable but generally positive, with intermediate densities often promoting the highest growth rates in moisture limited environments but also decreased photosynthetic pigments (Okland and Okland, 1996; Pederson *et al.* 2001). Increasing shoot density can help mats better retain moisture but also decreases the light levels available at depth due to shading and increased competition for the available light (Okland and Okland 1996; Tobias and Niinemets, 2010; van der Hooven and During, 1997). This decrease in available light at depth can cause an upwards shift in the level below the surface at which the moss shoots become unproductive and begin decomposition, decreasing photosynthetic capabilities of shoots and concentrating photosynthetic pigments in upper shoot segments (Tobias and Niinemets, 2010).

Temperature constraints exist on both ends of the spectrum, low temperatures can freeze shoots and decrease photosynthesis rates after thawing (Bjerke *et al.* 2013), while high temperatures increase evaporation rates and can place moisture stress on mosses (Busby *et al.* 1978; Dilks and Proctor, 1979; Skre and Oechel 1981). Species commonly have optimal temperatures between 15-25˚C, though Furness and Grime (1982) found that shoots grew from 5-30˚C and that rapid growth was still seen at the lower end of that spectrum, while temperatures above 30˚C often resulted in plant mortality. The optimal temperatures for moss growth are often lower than for vascular plants found in the same

areas, which is presumed to be an adaptation to aid moss growth over shoulder seasons (spring and fall) when moisture is less limiting but temperatures are also lower (Furness and Grime, 1982). Some moss species exhibit increases in maximum photosynthesis rates with seasonal in air temperature, often reaching peak rates in the summer months, and in general it has been found in a variety of forest types that higher soil and mean air temperature are good predictors of increases in above ground net primary productivity (Davey and Rothery, 1996; Vogel *et al.* 2008). The overall higher rates of C fixation due to increased mean air temperature is both direct and indirect, through warmer days and longer growing seasons which increased the number of potentially photosynthetic hours, as well as via increased microbial activity which allows for greater nutrient availability which can otherwise be a limiting factor to productivity in forests (Vogel *et al.* 2008).

Energy allocation in plants is divided between structural and photosynthetic components, with reports of photosynthetic pigment concentrations negatively correlated with allocation to non-photosynthetic stem tissue (Jägerbrand, 2005, 2012; McCall and Martin, 1991; Rice, 1995). Thus, knowledge of the concentrations of the various photosynthetic pigments in plants can be useful to address changing resource allocation strategies of plants in contrasting microclimate conditions. The most abundant pigments within terrestrial plants are chlorophylls and carotenoids, both so common due to their key roles in photosynthesis and photo-protection (Czeczuga, 1987; Fu *et al.* 2012; Wrolstad *et al.* 2005).

Chlorophylls are green pigments involved in photosynthetic light harvesting and energy transduction present within reaction centers, and come in two main forms: the primary pigment chlorophyll a (Chl *a*) and the accessory chlorophyll b (Chl *b*)

(Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001). Chl *a* is found in photosystems I and II, within the reaction centers, and within the pigment antenna (Lichtenthaler and Buschmann, 2001; McCall and Martin, 1991). Chl *b* is found only in the pigment antenna. The mass ratio of Chl a:b is essentially constant within photosystem I, but in photosystem II it can change depending on habitat light intensity and it is this change which is reported in studies (Lichtenthaler and Buschmann, 2001; McCall and Martin, 1991). A decrease in the ratio of Chl *a:b* represents an increase in the antenna system size within photosystem II (Lichtenthaler and Buschmann, 2001; McCall and Martin, 1991; Marschall and Proctor, 2004). It has been suggested that changes in the Chl a:b ratio in plants on the forest floor may also be due to increasing Chl b preferentially, as it can best absorb the photons at the forest floor after the tree canopies absorb more of the light within the Chl a absorption spectrum (Boardman, 1977). Average concentrations of between 1-3mg chl/g dry weight have been reported for feather mosses (Raeymaekers and Glime, 1986). Mosses typically have Chl a:b ratios ranging from 1.5-3, and as shade plants these values are much lower than typical for vascular plants (Marschall and Proctor, 2004; Martin and Churchill, 1982; Tobias and Niinemets, 2010).

Carotenoids are divided primarily into two groups: oxygen-free carotenes and oxygen-containing xanthophylls (Lichtenthaler, 1987). Carotenoids are found within photosystem II, and prevent the photosynthetic deactivation of reactive oxygen species and the reduction of their formation during times of high irradiance (Fu *et al.* 2012). Carotenoids are a group of pigments which are responsible for the red and yellow colours seen in plants, especially on shoulder seasons, and the mass ratio of total chlorophylls to carotenoids can be used to assess and compare the relative “greeness” of plant specimens

(Lichtenthaler and Buschmann, 2001; Wrolstad *et al.* 2005). This group of pigments is thought to be used most for photo-protection by plants in high light environments, though potentially they can also help to enable plants to intercept a maximum amount of light when in a light limited environment (Czeczuga 1987; Fu *et al.* 2012).

Changing pigment concentrations can enable plants to intercept a maximum amount of light when it is a limited resource, potentially increasing rates of photosynthesis, and this relationship has been found to hold for mosses which decrease their pigment concentrations on a dry mass basis with increasing levels of habitat light (Lichtenthaler *et al.* 2013; López and Carballeira, 1989; Hájek *et al.* 2009; Tobias and Niinemets, 2010). However, a range of responses have been found for the ratio of Chl *a:b* and the concentration of carotenoids, with previous studies reporting instances of increases, decreases, or a total lack of a response (Tobias and Niinemets, 2010; Hájek *et al.* 2009; Lopez and Carballeira, 1989; Rice et al. 2008). The ratio of total chlorophylls to carotenoids is reported to be negatively correlated with habitat irradiance as chlorophylls breakdown faster than carotenoids in situations of stress, damage, or senescence (Lichtenthaler and Buschmann, 2001; Tobias and Niinemets, 2010). Alterations of pigment contents in mosses is thought to be more dependent on moisture content than changes seen in vascular plants, as periods of active photosynthesis for mosses are often during times of low light intensity after water input events (Hájek *et al.* 2009).

Harvesting has been found to negatively correlate with feather moss species presence by a number of studies, and the decrease in ground cover has been attributed to the presumed negative response of common boreal moss species to the new set of microclimate conditions (Åström *et al.* 2007; Bergstedt *et al.* 2008; Marschall and

Proctor, 2004; Nelson and Halpern, 2005), though these often rely on changes to percent ground cover as opposed to quantifying the fitness of the shoots themselves. Feathermosses have been shown to be more productive in shadier areas, while *Sphagnum* is comparatively better suited to more open environments as it can maintain photosynthesis for longer after water input (Busby and Whitfield, 1978; Skre *et al.* 1983; Bisbee *et al.* 2001). Seasonal changes have been found in maximum photosynthesis rates for feathermoss species, with peaks in August when resource allocation is shifted from stem growth to pigment creation (Jägerbrand *et al.* 2012). Studies have reported contrasting results among feathermosses and *Sphagnum*, with some suggesting maximum photosynthetic rates were greater in *Sphagnum* (Goulden and Crill, 1997) and other finding greater rates in feathermosses (Bergeron *et al*. 2009), which highlights the site specific nature of this relationship. In terms of pigment contents, previous studies have found a positive correlation between photosynthesis rates and chlorophyll content in mosses (McCall and Martin, 1991; Gaberščik and Martinčič, 1987), though this relationship has been insignificant in other studies (Davey and Rothery, 1996).

Given the findings of Bisbee *et al.* (2001) that NPP from bryophytes was comparable or greater than over story NPP in a black spruce boreal forest, the impact of anthropogenic activities may have on the mosses of the forest floor are highly important. This present thesis sought to better assess the productivity of common moss species in a regenerating boreal forest, in order to help better the understanding of the longer-term effects of clear-cutting on local moss species. The first study specifically set out to determine the CO_2 fluxes resultant of photosynthesis and respiration from moss stems in natural field conditions. The experiment compared fluxes of samples taken from exposed

post-harvest forest sections, as well as along the edge between the post-harvest area and the unharvested forest, and the undisturbed mosses in the unharvested forest interior. Additionally, a comparison was done of the water holding capacities, moss mat densities, and monthly biomass increases of moss shoots from both the post-harvest areas and the unharvested forest. The second group of experiments set out to assess light responses and photosynthetic pigments of the same moss species across regenerated and unharvested areas of the forest over the 2015 growing season. This study was performed in a boreal black spruce forest in Pynn's Brook, Newfoundland. Blocks of the forest had been harvested for a prior study in 2003, and allowed to regenerate.

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Chapter 2: Seasonal productivity of mosses along a harvest gradient

2.1 Introduction

Globally, the boreal forest encompasses 27% of the total forested land and 40% of the terrestrial carbon (C) pool (Jonsson *et al.* 2015; Benscoter and Vitt, 2007). The boreal ecosystem is an economically and biologically important resource across Canada, and a better understanding of C pathways, sources, and sinks is needed to more accurately assess potential impacts of forestry activities and better prepare for a range of potential future climate patterns (Dussart and Payette, 2002; Lee *et al.* 2002; Swanson and Flanagan, 2001). In an undisturbed system, the C balance is the difference between the C used by plants for photosynthesis and that lost through respiration; an imbalance in these fluxes means that C is either stored within a system, which is then called a sink, or released into the atmosphere, when it is termed a source (Swanson and Flanagan, 2001). Up to 80% of the boreal forest area harvested in Canada is done by clear-cutting, which can lead to changes in ecosystem C fluxes due to increased temperatures, greater levels of incoming irradiance, more air movement, and a decrease in moisture; additionally a new set of environmental conditions is also created along the edge of any adjacent unharvested forests (Caners *et al.* 2010; Hart and Chen, 2006; Nelson and Halpern, 2005; Youngblood and Titus, 1996). This altered set of environmental conditions is often present for many years after harvesting, and shifts in C fluxes due to the impacts of tree harvesting on flora can greatly affect an ecosystems ability to remain a net C sink (Gorham, 1991).

Within the boreal forest, ground cover is often predominantly bryophytes, and their net CO_2 exchange can account for up to half of the total ecosystem exchange

(Bisbee *et al*. 2001; Benscoter and Vitt, 2007; DeLucia *et al*. 2003; Bona *et al.* 2013). Estimates suggest that up to 80% of the terrestrial C pool found in boreal systems can be attributed to biomass inputs from the forest floor bryophyte community, composed mainly of lichens and mosses, but the long-term effects of harvesting on these small plants is often overlooked in large scale site assessments (Benscoter and Vitt, 2007). In addition to their contribution to the C fluxes of a habitat, mosses within boreal systems are known to regulate a range of abiotic conditions, such as soil temperature, soil moisture, and nutrient cycling (Bisbee *et al.* 2001; Hart and Chen, 2006; Jonsson *et al.* 2015; Kolari *et al.* 2006; Müller *et al.* 2016; Turetsky *et al.* 2012). Mosses are commonly thought to be most regulated by the moisture regime within their habitat, and if their moisture needs are met then light and temperature are the next most common abiotic determinants of moss productivity (Bergeron *et al.* 2009; Botting and Fredeen, 2006).

Mosses are useful indicators of changing microclimate conditions within disturbed ecosystems as they are poikilohydric organisms that lack true roots, used to suck up water from depth, and stomata, used to regulate water loss to the atmosphere (Jonsson *et al.* 2015; Proctor and Tuba, 2002; Tobias and Niinemets, 2010). The lack of water regulation mechanisms leaves mosses susceptible to drying and at low water contents moss shoots cease metabolic activity, therefore they characteristically inhabit shady and damp areas where evaporation is lower and they can be more productive (Proctor, 1990; Tobias and Niinemets, 2010; Turetsky *et al.* 2012). Mosses that grow in boreal ecosystems commonly grow in dense mats or cushions, which aids in water retention after rain events (Proctor, 1990). Water is lost more readily through evaporation due to higher vapour pressure deficits in areas with increased temperatures and incoming solar radiation, such

as a forest post-harvest, an effect which can potentially reduce the periods of active photosynthesis for mosses (Hylander *et al.* 2005; Palviainen *et al.* 2005; Wagner *et al.* 2012). These high radiation environments can also have adverse effects on the productivity of mosses via photo-inhibition, as many shade-dwelling species lack adequate sun protection (Arsenault *et al.* 2012; Mishler and Oliver, 2009). Mosses cope with moisture stress via to a unique cellular structure that allows many species to rapidly recover from drought periods; the cytoplasm in moss cells can exist for long periods of time without water inputs and has the ability to regain metabolic function upon rewetting, though this often comes with a spike in respiration rates and recovery can be hindered by increased air temperatures (Turetsky *et al.* 2012; Proctor, 1990). As moisture needs can vary greatly between species, there can be a variety of responses to harvest events, with the most commonly reported changes seen in the growth rates, productivity, or distribution patterns in a given area, all of which can be measured to better assess impacts of natural and anthropogenic disturbances (Bansal *et al.* 2012; Bu *et al.* 2011; Gignac, 2001). Alternatively, moss mats can also respond to changing environmental conditions by increasing or decreasing the density of moss shoot packing to help better retain moisture, which can have both positive and negative effects of individual shoot productivity (Bates, 1988; Okland and Okland, 1996; Tobias and Niinemets, 2010).

The ground layer of boreal black spruce forests are typically covered by *Sphagnum* and weft forming feathermoss species, among the most common are *P. schreberi*, *H. splendens*, and *P. crista-castrensis* (Swanson and Flanagan, 2001; Bates and Farmer, 1992; Rice *et al.* 2011; Benscoter and Vitt, 2007). In some boreal ecosystems the forest floor bryophytes contribute from 10-50% of the net primary productivity of an area

(Bisbee et al. 2001), yet little is known about the long-term changes to their productivity after clear-cutting, especially in a cold maritime environment such as the island of Newfoundland. Other studies on moss productivity have focused on longer time periods and on photosynthesis per unit ground area as opposed to at the stem level, or instead measured the proportional ground cover of feathermoss species after harvest events (Bisbee *et al.* 2001; Caners et al. 2010; Dussart and Payette, 2002; Swanson and Flanagan, 2001). All three feathermoss species included in this study are ectohydric pleurocarpous mosses that prefer moist shady areas, with water needs being met through precipitation and ambient air moisture (Bates and Farmer, 1992; Okland and Okland, 1996; Rice *et al.* 2008). *Sphagnum* is common to the study area, but is known to grow often in more exposed and brighter spaces (Bisbee *et al.* 2001). The objectives of this study were to assess 1) recovery of photosynthesis in *Sphagnum* and feathermosses a decade after clear-cutting in black spruce stands on the island of Newfoundland, and 2) the post-harvest impacts on moss stem density and growth in response to ground disturbances or light availability.

2.2 Materials and Methods

2.2.1 Site description

The study site (lat. 48^{o} 53’ 14’N, 63^{o} 24’08’W) was located near the town of Pasadena, Newfoundland and Labrador, Canada within the Maritime Low Boreal Ecoclimate region (Lbm) of the Ecoregions working group (1989). Average annual rainfall from 1981-2010 was 727 mm, and average annual temperature was 4.6 ^{o}C (Government of Canada, 2016). Average monthly rainfall measured over the growing

season at the nearby Deer Lake climate center ranged from 33.6 mm in August to 74.1 mm in June. The site was adjacent to a riparian zone for a small stream, along that edge the soil was an Orthic Gleysol, transitioning to a Ferro-Humic Podzol as the site was traversed. The organic layer thickness ranged from 6-15cm in depth (Moroni and Zhu, 2012).

The study site was composed of eight 50 m x 50 m blocks, of which four were clearcut in 2003 and allowed to regenerate, and four were left intact as black spruce dominated control plots (Moroni *et al.* 2009). The most common ground cover bryophyte species in both the post-harvest and the unharvested blocks were *H. splendens*, *P. schreberi*, *P. crista-castrensis*, and *Sphagnum*, though the post-harvest blocks also had a larger proportion of ground covered by woody debris. In this study, the experimental site was further divided into three types of moss habitats for sampling based on ground level light availability measured as leaf area index (using LAI-2200 Canopy Analyzer; Li-Cor Biosciences, Lincoln, NE, USA): open areas of the low-density spruce regenerated after clear-cut (LAI: 0.97±0.55); along the forest edge (LAI: 2.68±0.18), and within the unharvested forest blocks (LAI: 4.78±0.28) (Figure 2-1).

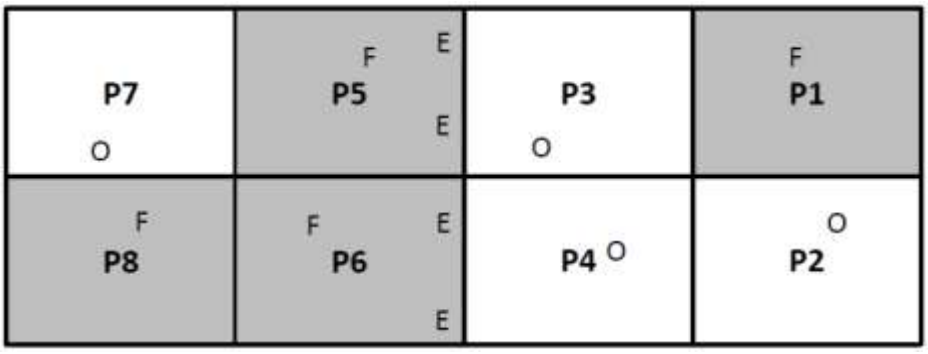

Figure 2-1. Experiment block and sampling location design for Pynn's Brook site. Clear-cut blocks are P2, P3, P4, and P7 (white fill). Post-harvest open sampling locations indicated by the letter "O". Unharvested blocks are P1, P5, P6, and P8 (grey fill). Interior forest sampling is identified by the letter "F", and forest edge sampling locations by the letter "E".

2.2.2 In situ photosynthesis

Field measurements were carried out on light rainy/misty days roughly every 7-10 days from June to October, 2015. Four green moss shoots of each species were collected from the 12 sampling sites (Figure 2-1), and cut to 2 cm length from the tip (*H. splendens* samples were cut to the base of the uppermost leaf segment). Net photosynthesis and dark respiration were measured using a Li-6400 XT portable photosynthesis system equipped with LI-6400-24 Bryophyte Chamber (Li-Cor Biosciences, Lincoln, Nebraska, USA). During the measurements (2 min of photosynthesis with light and 2 min of dark respiration), the chamber environment was set to mirror the ambient climate as much as possible (ambient PAR level, 16-20 ^{o}C temperature, 75% RH, 390 ppm CO_2). Immediately after the measurements of photosynthesis, samples were sealed in plastic bags to limit water loss, and transported to the laboratory to determine fresh and dry weight (dried for 48 hours at 75˚C). Net photosynthesis and dark respiration were recalculated on a dry-weight basis using the algorithm provided with LI-6400-24

Bryophyte Chamber. Gross photosynthesis was obtained by adding respiration rates to net photosynthesis. At each sampling spot concurrently with moss gathering, measurements were collected for: ambient PAR (LI-189 Light Sensor), soil temperature at 5 cm depth and volumetric soil moisture content at 5 cm depth (Campbell Hydro-Sense penetration probe). During the growing season, at the center of each block, air temperature, soil temperature (5cm depth), relative humidity, soil moisture content (5 cm depth) and photosynthetically active radiation (PAR) were also continuously recorded with a HOBO Micro-Station (OnSet Computer Corporation, Massachusetts, USA). Ground level leaf area index was measured for each sampling plot with a LAI-2200 Canopy analyzer (Li-Cor Biosciences, Lincoln, Nebraska, USA) in late August.

2.2.3 Photosynthesis light response curve

Photosynthetic light response curves were calculated for each of the four moss species from the post-harvest and the unharvested blocks. In late August, small sections of moss mats were collected from the ground layer in the sampling plots, transported to the laboratory, and placed in a Conviron Doirma plant growth chamber (daytime: 12 hours at 14ºC, >75% RH, ~100 µmol m^{-2} s^{-1}light intensity; night: 12 hours at 12ºC, >75% RH, complete darkness) to acclimate for 2-3 days prior to the light response experiments. On the measurement day, fully-hydrated moss shoots were cut 2 cm length from tips (to the base of the upper leaf segment for *H. splendens*) and placed in the bryophyte chamber of the Li-6400XT to record rates of net photosynthesis with decreasing PAR (600, 500, 400, 300, 200, 100, 75, 50, 25, and 0 µmol m^{-2} s^{-1}) following the methods of Wang *et al.* (2016) and McCall and Martin (1991). Moss shoots in the bryophyte chamber were

allowed to acclimatize for 2 minutes at each step of light intensity before a net photosynthesis reading was recorded. The resultant photosynthetic light response data was curve fitted with a non-rectangular hyperbolic function (Farquhar and Wong, 1984):

$$\theta(A + R_d) - (\varepsilon Q + P_{max})(P_{max} + R_d) + \varepsilon Q P_{max} = 0 \quad (1)$$

where Q is the incoming radiation, PAR (μmol m^{-2} s^{-1}), P_{max} is the maximum gross photosynthesis rate at the point of light saturation (mmol CO_2 g^{-1} s^{-1}), ε, the initial curve-slope, is the apparent quantum efficiency, θ is a measure of convexity of the response curve, and R_d is the dark respiration rate (mmol CO_2 g^{-1} s^{-1}).

2.2.4 Photosynthesis dehydration curves

Responses of photosynthesis to dehydration were tested in the laboratory in an attempt to determine the duration of photosynthesis for moss shoots after wetting, in addition to species and treatment specific optimal water contents. In late July, small moss patches collected from the harvested and unharvested blocks were transported to the growth chamber in the laboratory for 24 h of acclimation, as was done with light response experiment. Moss shoots were cut to 2 cm length from the tip (*H. splendens* samples were cut to the base of the uppermost leaf segment), and saturated for 1h prior to measurements. The initial fresh weights of fully-hydrated moss shoots were recorded after gently shaking off excess water and net photosynthesis was measured in the Li-6400XT. This process of weighing and measuring was repeated every 30 minutes, until net photosynthesis was almost zero, with mosses left to dry outside of the Li-Cor

bryophyte chamber between readings. At each time step, measurement took 4 min (2 min for net photosynthesis and 2 min for dark respiration). During the photosynthesis measurement, PAR in the bryophyte chamber was set at 500 µmol $m^{-2}s^{-1}$, an irradiance level above light saturation point for all moss species tested (Rice *et al.* 2011). The rest of the environmental parameters were identical to the settings used for the light response experiment described earlier. At each level of shoot water content (g fresh weight/g dry weight, g g^{-1}), net photosynthesis rates were expressed as percentage of maximum photosynthesis.

2.2.5 Shoot elongation and biomass growth

Shoot biomass growth was measured through the use of growth plates in the post-harvest and the unharvested blocks beginning in June 2015. In early June, small patches of each moss species were collected from natural turfs in both the treatments and brought into the lab. Sixteen shoot segments of a species were cut to the same length as for photosynthetic tests, and were planted in small holes drilled in a 4 cm x 4 cm grid pattern on clear plastic plates, the lower end of shoot density in natural moss turf. *Sphagnum* was planted but didn't survive the low-density plantation test because of quick dehydration of the tissues at such a low density. Ten plates of each species were inserted into the natural moss turf in each of the four post-harvest and the four unharvested blocks. Fifty extra shoots of each moss species were cut to 2 cm length at the time of planting for an initial baseline of dry weight/shoot. The measurements of biomass were repeated monthly when two plates of each species were harvested from each of the 8 blocks.

2.2.6 Statistical analysis

Net and gross photosynthesis rates were analyzed using linear mixed models to assess differences in rates between the three treatments and among species over the growing season. The study represented a randomized experiment design with repeated measures. The photosynthesis rates involved two factors: treatment/sub-treatment (post-harvest open, unharvested forest edge, and unharvested forest interior) and measurement day. The model contained the fixed effects of treatment and day, the random effect of blocks nested in the treatment, and a statement to account for the repeated nature of the experiment. An additional model was run for net and gross photosynthesis rates that included species as an effect in addition to treatment and sampling date. Differences of least square means (lsmeans) was used to test for differences among species at each treatment/sub-treatment level ($p<0.05$). Seasonal microclimate measurements and biomass increases were compared between treatment levels using the same mixed models and differences of lsmeans analysis as was used for photosynthesis rate comparisons. Optimal water content data, natural stem density, and specific leaf area measurements were compared by using differences of lsmeans. The type I error threshold was fixed at $\alpha=0.05$. All statistical analyses were performed using SAS version 9.3. Light response curves were modelled using SigmaPlot 11.0.

2.3 Results

2.3.1 Environmental conditions

Daytime PAR in the post-harvest blocks was often 10x greater than PAR readings recorded in the unharvested blocks as measured from the center of each test block (Figure 2-2). Hourly air temperatures measured by the dataloggers peaked in August and were

lowest in October for both treatments, and air temperature from sunrise to sunset was significantly higher in the post-harvest blocks than in the unharvested blocks ($p<0.0001$; Figure 2-2). The vapor pressure deficit (VPD) was greater in the post-harvest blocks than in the unharvested blocks ($p=0.0141$), especially during the middle of growing season (Figure 2-2). Seasonal means of VPD were 296.90 ± 5.81 Pa in the unharvested blocks and 318.29 ± 6.49 Pa in the post-harvest blocks. Similar differences in levels of incoming light measurements were found on test days as well; at the time of sampling on rainy days incoming solar radiation was about 450 µmmol m^{-2} s^{-1} at its peak in the open ground compared to less than 50 µmmol m^{-2} s^{-1} in the unharvested blocks (Figure 2-4).

Over the growing season, as measured on the 15 test days, soil temperature at 5 cm depth ranged from 7-16 ˚C with peaks for all three treatments in August (Figure 2-4). Soil temperature readings on measurement days decreased in the order: post-harvest open, forest edge, and forest interior; a model of soil temperature in all three treatments had highly significant effects of treatment and sampling date ($p<0.0001$), and the interaction between treatment and sampling date was significant as well ($p<0.0001$) (Figure 2-4). This trend was supported by the daily means measured by the dataloggers over the season in post-harvest and unharvested blocks, as soils were always warmer in the post-harvest blocks than the unharvest blocks (Figure 2-3). Soil moisture as measured on sampling days was greater in open post-harvest sample areas, and measurements made along the edge and within the unharvested blocks were often similar (Figure 2-4). The soil moisture analysis detected a treatment effect ($p=0.0002$) and an effect of sampling data ($p<0.0001$), but no interaction between treatment and sampling date ($p=0.0796$) (Figure 2-4). The daily means of soil moisture as measured by the dataloggers supported these findings, as

soils were often wetter in the post-harvest sampling blocks than in the unharvested blocks (Figure 2-3). Overall, soils were warmer and wetter at 5cm depth in the post-harvest blocks than in the unharvested blocks (Figure 2-3).

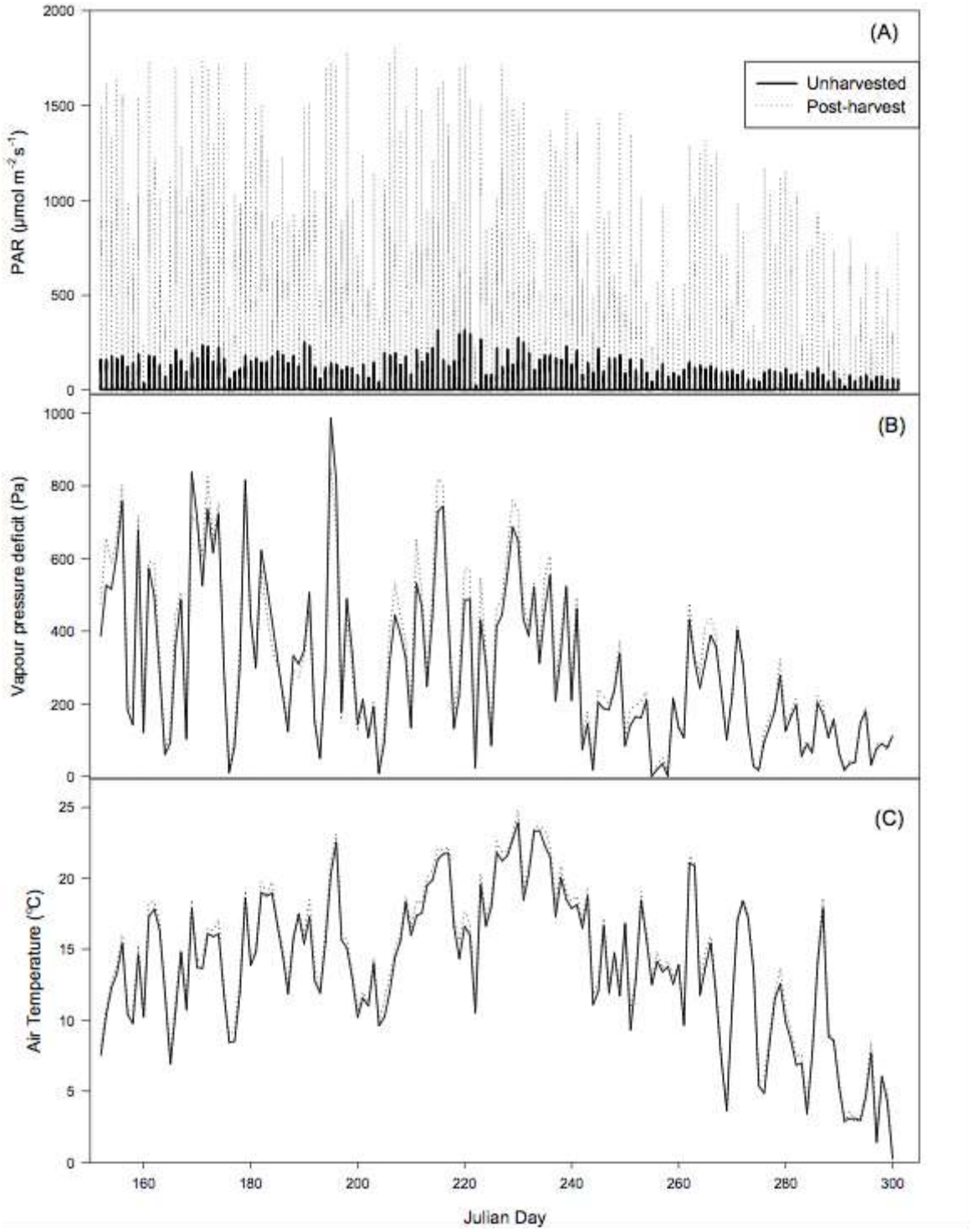

Figure 2-2. Daily averages of A) values from sunrise to sunset of photosynthetically active radiation (PAR) B) sunrise to sunset daily air temperature (°C) and C) 24hour

averages of vapor pressure deficit (VPD) over the 2015 growing season, as measured by data loggers placed in the center of 4 post-harvest and unharvested forest blocks.

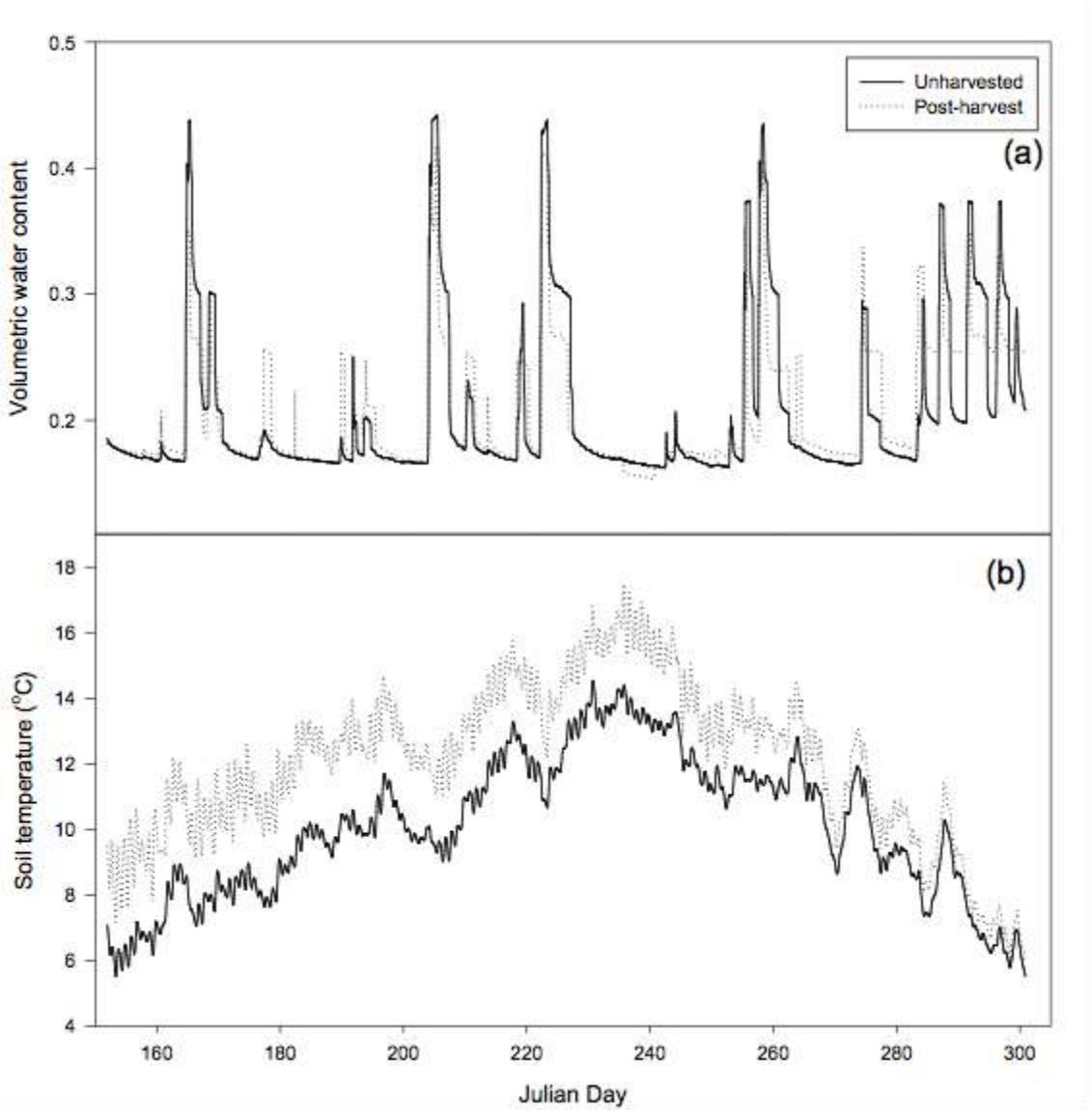

Figure 2-3. Daily averages of A) soil volumetric water content (at 5cm depth) and B) soil temperature (at 5cm depth) over the 2015 growing season, as measured by data loggers placed in the center of 4 post-harvest and unharvested forest blocks.

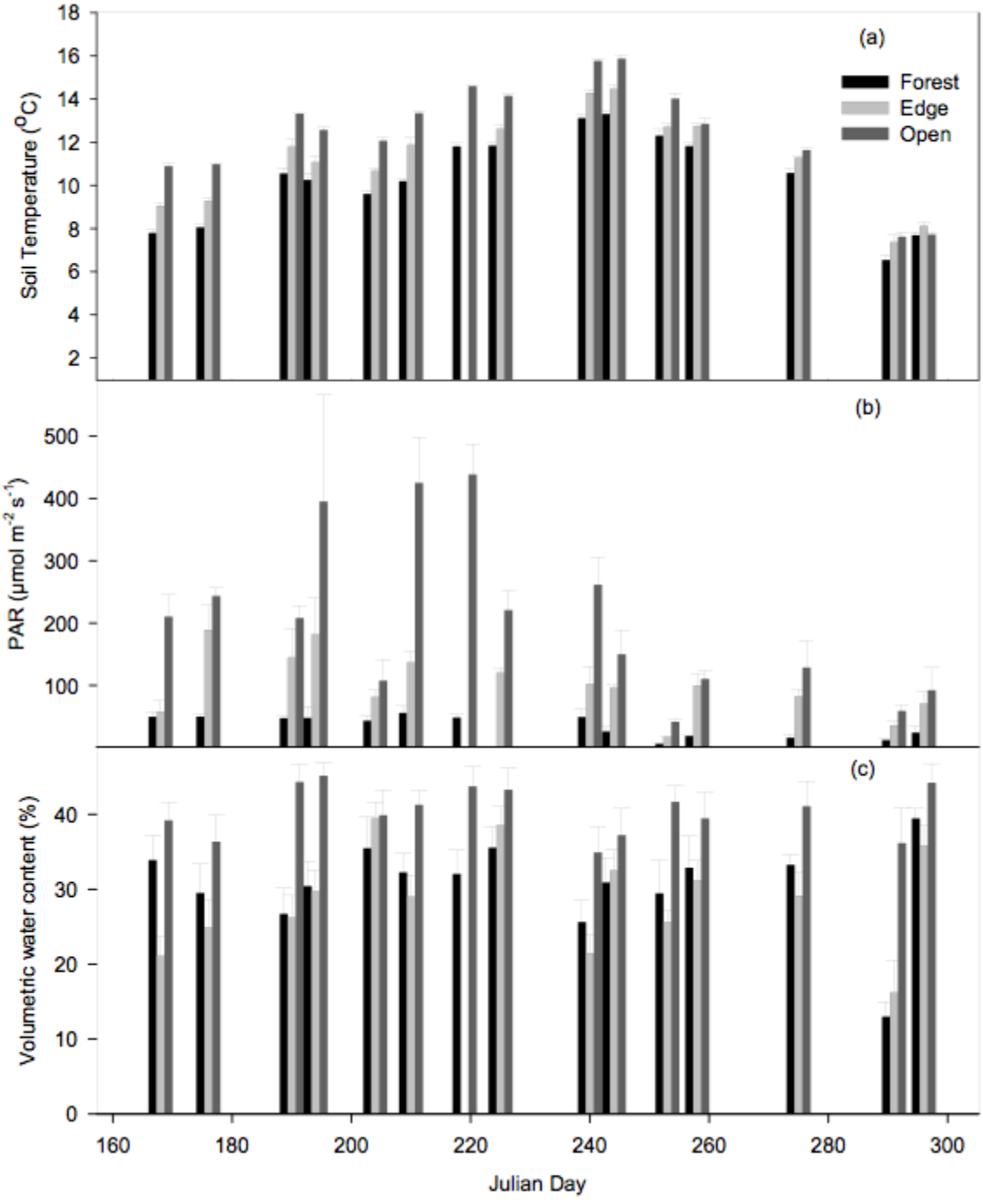

Figure 2-4. Average microclimate readings for each harvest treatment level (open: post-harvest; edge: unharvested forest edge; forest: unharvested forest interior) measured in conjunction with carbon flux readings over the 2015 growing season. A) Average soil

temperature (with SE). B) Average instantaneous PAR readings (with SE). C) Volumetric soil moisture (with SE).

2.3.2 Photosynthesis

The instantaneous moss photosynthesis was measured only on rainy and/or misty days to ensure that moss shoots were photosynthetically active. The light intensities used in the bryophyte chamber were set to mirror the ambient light levels at each of the individual sampling plots scattered in the forest blocks, along the forest edge, and across the open areas of the post-harvest blocks. Instantaneous PAR readings gathered at the time of sample collection were significantly different among treatments ($p<0.0001$); readings were always highest in open post-harvest samples sites and always lowest in interior forest areas, with values measured at the forest edge in between (Figure 2-4). Measurements of PAR used for photosynthesis tests ranged from 41-438 µmol m^{-2} s^{-1} in the post-harvest open sites, from 17-189 µmol m^{-2} s^{-1} at the forest edge, and from 6-56 µmol m^{-2} s^{-1} in the interior forest (Figure 2-4). The fresh to dry weight ratios were similar between treatments for all species, with averages ranging from 5.5-7.5 g g^{-1} for all three feathermosses, and from 12-14 g g^{-1} for *Sphagnum* samples (Figure 2-5). All water contents measured for the samples used for photosynthesis measurements were within an acceptable range over the season based on the drying curves created for species from both post-harvest and unharvested forest blocks.

For all four moss species, negative rates (net emission of CO_2, respiration rates greater than gross photosynthesis rates) of net photosynthesis changed to positive (net assimilation of CO_2, gross photosynthesis rates greater than respiration rates) in late June, and continued to be above 0 in all treatments with few exceptions (Figure 2-6). Rates were always smaller or more negative in samples from the forest interior than samples

from the post-harvest blocks or the forest edge, but this was not a significant difference over the season (Table 2-1 & Figure 2-6). Within treatments, there was no significant difference in net photosynthesis among species (Table 2-1 & 2-2). A treatment effect on net photosynthesis was found only for *Sphagnum* and not for the feathermosses (Table 2-4 & Figure 2-6). The sampling date had a significant effect on net photosynthesis for all species, and there was a significant interaction between sampling date and treatment for *H. splendens* and *P. schreberi* (Table 2-4). When all species were grouped together, net photosynthesis rates were significantly related to the day of sampling, the species, and the interaction of treatment and sampling date (Table 2-4).

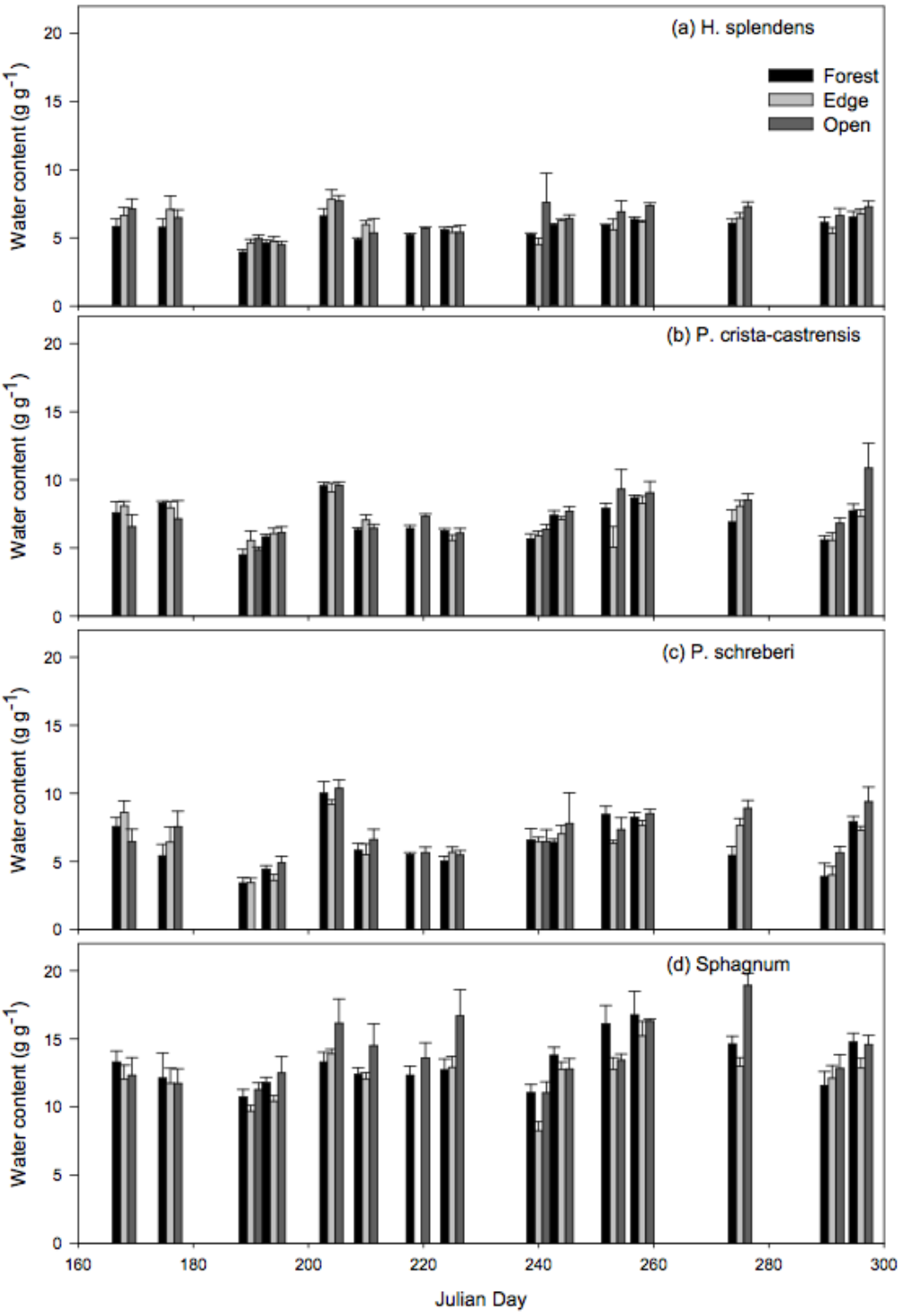

Figure 2-5. The measured field water contents (with standard error) for samples of A) *H. splendens*, B) *P. crista-castrensis*, C) *P. schreberi*, and D) *Sphagnum* from the forest

interior, forest edge, and open areas of post-harvest sites as measured on rainy days over the 2015 growing season. (n=4).

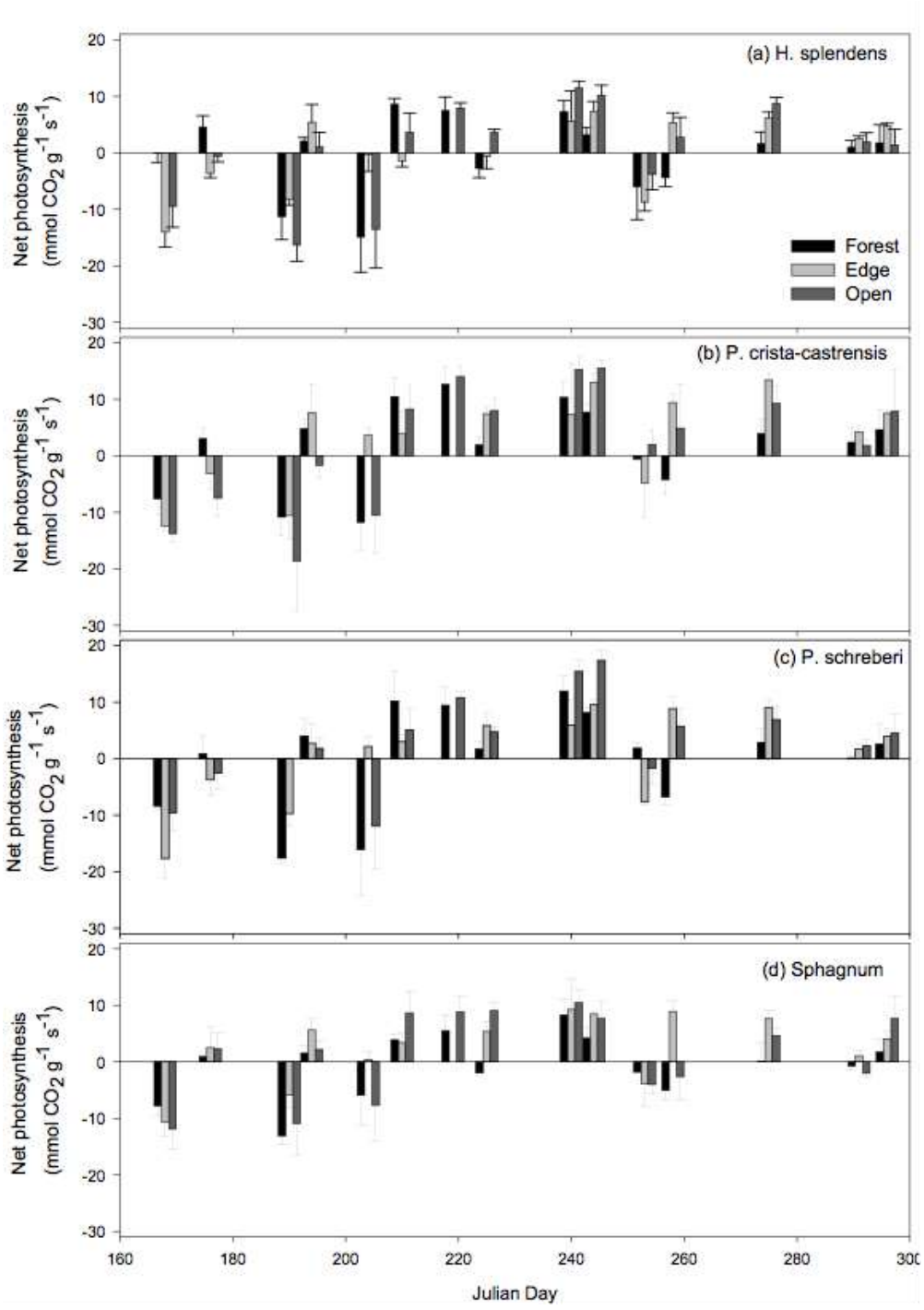

Figure 2-6. The net photosynthesis rates (with standard error) for samples of A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* from the forest interior, forest edge, and open areas of post-harvest sites as measured on rainy days over the 2015 growing season. Net photosynthesis rates were

measured using light intensities individual to each sampling location, as measured at time of collection. (n=4)

Adding dark respiration to net photosynthesis, we found that the instantaneous gross photosynthesis was significantly greater for *P. crista-castrensis* than all other species ($p<0.05$) within each treatment, and there were no other significant differences among species within any treatment (Table 2-1 & 2-3). The gross photosynthesis rates were significantly greater in samples from the post-harvest blocks than samples from the forest interior for all species, while for *H. splendens* and *P. schreberi* the rates at the forest edge were significantly lower than rates of samples from the post-harvest blocks, and for *P. crista-castrensis* the rates found at the forest edge were significantly greater than for samples from the forest interior (Table 2-3 & Figure 2-7). For *Sphagnum* the greatest gross photosynthesis was measured in the open areas of previously harvested blocks and the lowest was measured in the unharvested blocks, and the seasonal rates of gross photosynthesis for *Sphagnum* from all three treatments were significantly different (Table 2-3 & Figure 2-7). A treatment effect was found for instantaneous gross photosynthesis rates for all three feathermosses as well as for *Sphagnum* (Table 2-4). Again, gross photosynthesis was significantly affected by sampling date for all moss species, but significant interaction between sampling date and treatments was only observed in *H. splendens* and *P. crista-castrensis* (Table 2-4). When all species were grouped together, instantaneous gross photosynthesis rates were significantly related to the treatment, the day of sampling, the species, and the interaction of treatment and sampling date (Table 2-4).

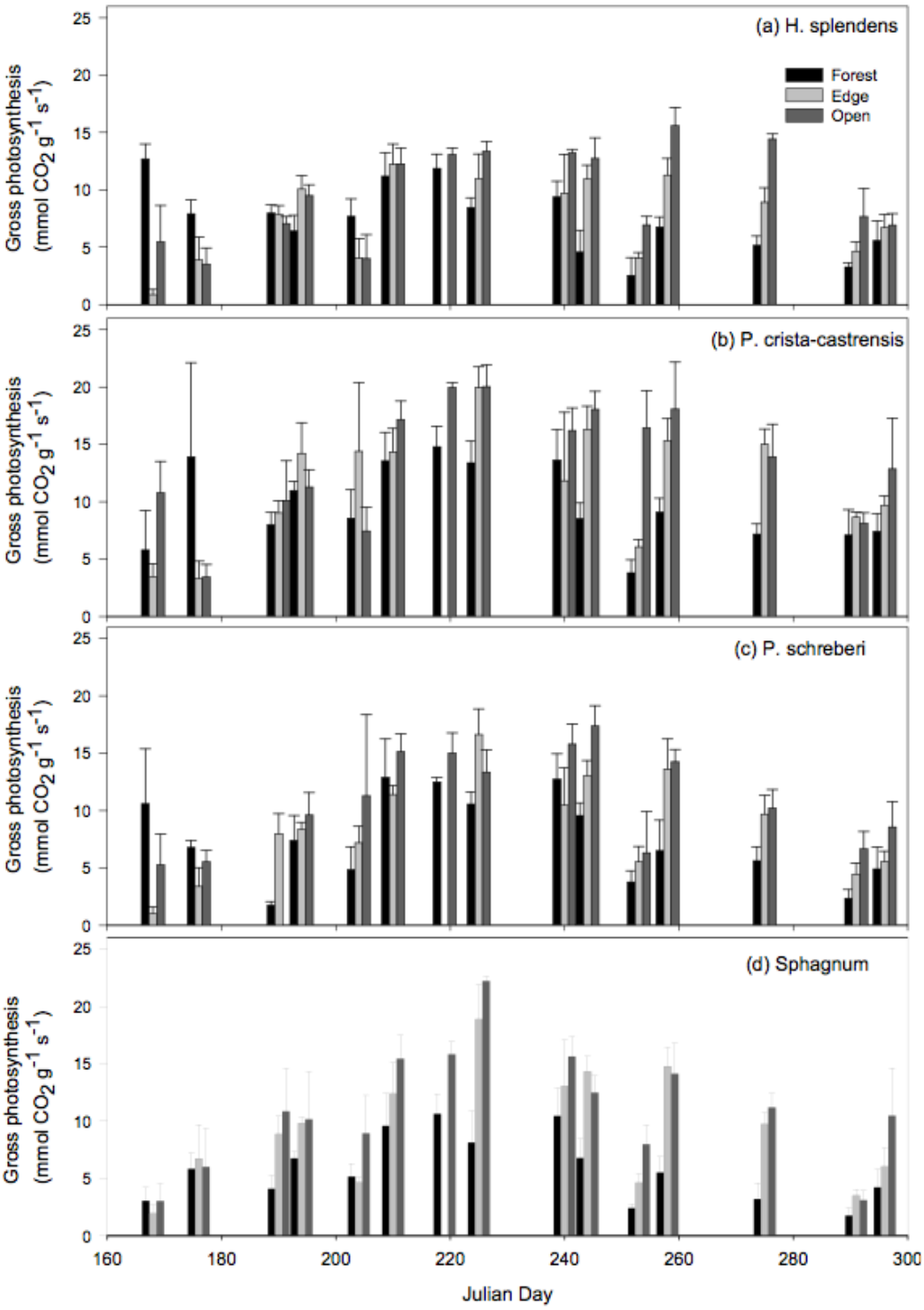

Figure 2-7. The gross photosynthesis rates (with standard error) for samples of A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D)

Sphagnum from the forest interior, forest edge, and open areas of post-harvest blocks as measured on rainy days over the 2015 growing season. (n=4).

Table 2-1. Seasonal means (with standard error in parentheses) of net photosynthesis, gross photosynthesis, and respiration (mmol CO_2 g^{-1} s^{-1}) for four moss species from June-November 2015 and collected from three harvest treatment levels (post-harvest blocks, along the edge of unharvested blocks, and within the interior of the unharvested blocks). (n=64).

Treatment	Species	Net photosynthesis	Gross photosynthesis	Respiration
Forest Interior	H. splendens	-0.225 (1.086)	7.498 (0.474)	-7.722 (1.006)
	P. crista-castrensis	1.799 (1.193)	9.674 (0.648)	-7.875 (0.995)
	P. schreberi	-0.036 (1.423)	7.433 (0.707)	-7.294 (1.254)
	Sphagnum	-0.397 (0.903)	5.941 (0.548)	-6.282 (0.717)
Forest Edge	H. splendens	0.338 (1.034)	7.739 (0.614)	-7.7349 (0.785)
	P. crista-castrensis	3.575 (1.337)	11.825 (0.906)	-8.250 (1.005)
	P. schreberi	1.706 (1.154)	8.772 (0.689)	-7.036 (0.866)
	Sphagnum	2.720 (0.976)	9.380 (0.801)	-6.644 (0.701)
Post-harvest	H. splendens	1.061 (1.254)	9.776 (0.630)	-8.706 (0.985)
	P. crista-castrensis	2.636 (1.725)	13.785 (0.853)	-11.148 (1.216)
	P. schreberi	2.704 (1.531)	9.914 (0.843)	-5.689 (1.123)
	Sphagnum	2.173 (1.201)	11.413 (0.889)	-9.223 (0.883)

Table 2-2. P values for differences of least square means analysis of seasonal net photosynthesis rates for four moss species from three sampling treatment sites (open areas of post-harvest blocks, along the edge of unharvested forest blocks, and within unharvested forest blocks) from a black spruce site over the 2015 growing season from June-October 2015. Dark gray represents comparisons among species within a given treatment, and light gray denotes comparisons among treatments for a given species. Significant differences are present if $p<0.05$.

	Species	H. splendens		P. crista-castrensis			P. schreberi			Sphagnum		
Species	Treatment	Edge	Open	Forest	Edge	Open	Forest	Edge	Open	Forest	Edge	Open
H. splendens	Forest	0.7556	0.4703	0.2516	0.0377	0.1101	0.9155	0.2879	0.0708	0.9231	0.1001	0.1784
	Edge		0.6914	0.4186	0.0831	0.2095	0.8377	0.4613	0.143	0.687	0.1934	0.314
	Open			0.6785	0.1725	0.3831	0.5416	0.7249	0.2755	0.4172	0.3584	0.5361
P. crista-castrensis	Forest				0.331	0.6402	0.3029	0.9589	0.4879	0.2177	0.6071	0.8338
	Edge					0.6118	0.0502	0.3191	0.7835	0.0312	0.6439	0.4466
	Open						0.1391	0.6132	0.818	0.0932	0.9632	0.7976
P. schreberi	Forest							0.3418	0.0914	0.8408	0.1272	0.2191
	Edge								0.4683	0.2512	0.5817	0.7986
	Open									0.0595	0.8535	0.6286
Sphagnum	Forest										0.0846	0.1529
	Edge											0.7621

Table 2-3. P values for differences of least square means analysis of seasonal gross photosynthesis rates of four moss species from three harvest treatments (open areas of post-harvest blocks, along the edge of unharvested forest blocks, and within unharvested forest blocks) from a black spruce site over the 2015 growing season from June-October 2015. Dark gray represents comparisons among species within a given treatment, and light gray denotes comparisons among treatments for a given species. Significant differences are present if $p<0.05$, and are marked with a *.

	Species	H. splendens		P. crista-castrensis			P. schreberi			Sphagnum		
Species	Treatment	Edge	Open	Forest	Edge	Open	Forest	Edge	Open	Forest	Edge	Open
H. splendens	Forest	0.8099	0.0213*	0.0264*	<.0001	<.0001	0.9478	0.206	0.001	0.1153	0.0582	<.0001
	Edge		0.0442*	0.0537	<.0001*	<.0001	0.7623	0.3159	0.0029	0.0756	0.1064	0.0003
	Open			0.9182	0.0451	<.0001*	0.0189	0.3233	0.3064	0.0001	0.6924	0.1007
P. crista-castrensis	Forest				0.0339*	<.0001*	0.0235*	0.3706	0.2576	0.0002*	0.7666	0.0786
	Edge					0.0563	<.0001	0.0034*	0.3319	<.0001	0.0173*	0.6869
	Open						<.0001	<.0001	0.0037*	<.0001	<.0001	0.0181*
P. schreberi	Forest							0.1877	0.0009*	0.1346	0.0521	<.0001
	Edge								0.0486*	0.0054	0.5516	0.0095
	Open									<.0001	0.1598	0.5562
Sphagnum	Forest										0.0006*	<.0001*
	Edge											0.0425*

Table 2-4. Linear mixed model analysis results (p values, significant values in bold) net and gross photosynthesis rates for *Hylocomium splendens*, *Ptilium crista-castrensis*, *Pleurozium schreberi*, and *Sphagnum* collected in post-harvest blocks, along the edge of an unharvested blocks, and within the interior of the unharvested blocks over the 2015 growing season (June – November). (n=64).

Species	Factor	DF	Net photosynthesis (*p* value)	Gross photosynthesis (*p* value)
H. splendens	Treatment	2	0.7326	**0.0102**
	Day	14	**<0.0001**	**<0.0001**
	Treatment x day	27	**0.0004**	**<0.0001**
P. crista-castrensis	Treatment	2	0.2998	**0.0370**
	Day	14	**<0.0001**	**<0.0001**
	Treatment x day	27	0.0914	**0.0301**
P. schreberi	Treatment	2	0.2280	**0.0227**
	Day	14	**<0.0001**	**<0.0001**
	Treatment x day	27	**0.0014**	0.2782
Sphagnum	Treatment	2	**0.0117**	**0.0027**
	Day	14	**<0.0001**	**<0.0001**
	Treatment x day	27	0.2297	0.2594
All species together	Treatment	2	0.1596	**0.0018**
	Day	3	**0.0051**	**<0.0001**
	Species	14	**<0.0001**	**<0.0001**
	Treatment x species	6	0.5121	0.0605
	Treatment x day	27	**<0.0001**	**<0.0001**
	Day x species	42	0.6113	0.7224
	Treatment x species x day	80	0.9999	0.7799

The maximum gross photosynthesis rates derived from the light response curves were higher in samples from the unharvested blocks for *P. schreberi* (post-harvest P_{max}= 13.62 ± 1.16 mmol CO_2 g^{-1} s^{-1}, unharvested P_{max}= 20.96 ± 2.63 mmol CO_2 g^{-1} s^{-1}; p=0.0169) and *H. splendens* (post-harvest P_{max}= 16.16 ± 1.81 mmol CO_2 g^{-1} s^{-1}, unharvested P_{max}= 18.85 ± 2.11 mmol CO_2 g^{-1} s^{-1}; p >0.05), while the opposite was true for *P. crista-castrensis* (post-harvest P_{max}= 17.69 ± 2.29 mmol CO_2 g^{-1} s^{-1}, unharvested P_{max}= 14.92 ± 0.70 mmol CO_2 g^{-1} s^{-1}; p>0.05) and *Sphagnum* (post-harvest P_{max}= 22.15 ± 1.78 mmol CO_2 g^{-1} s^{-1}, unharvested P_{max}= 18.30 ± 2.13 mmol CO_2 g^{-1} s^{-1}; p>0.05) (Figure 2-8). All of the test species from both treatments experienced a decline in photosynthesis rates above PAR values of 200-400 µmol m^{-2} s^{-1} (Figure 2-8).

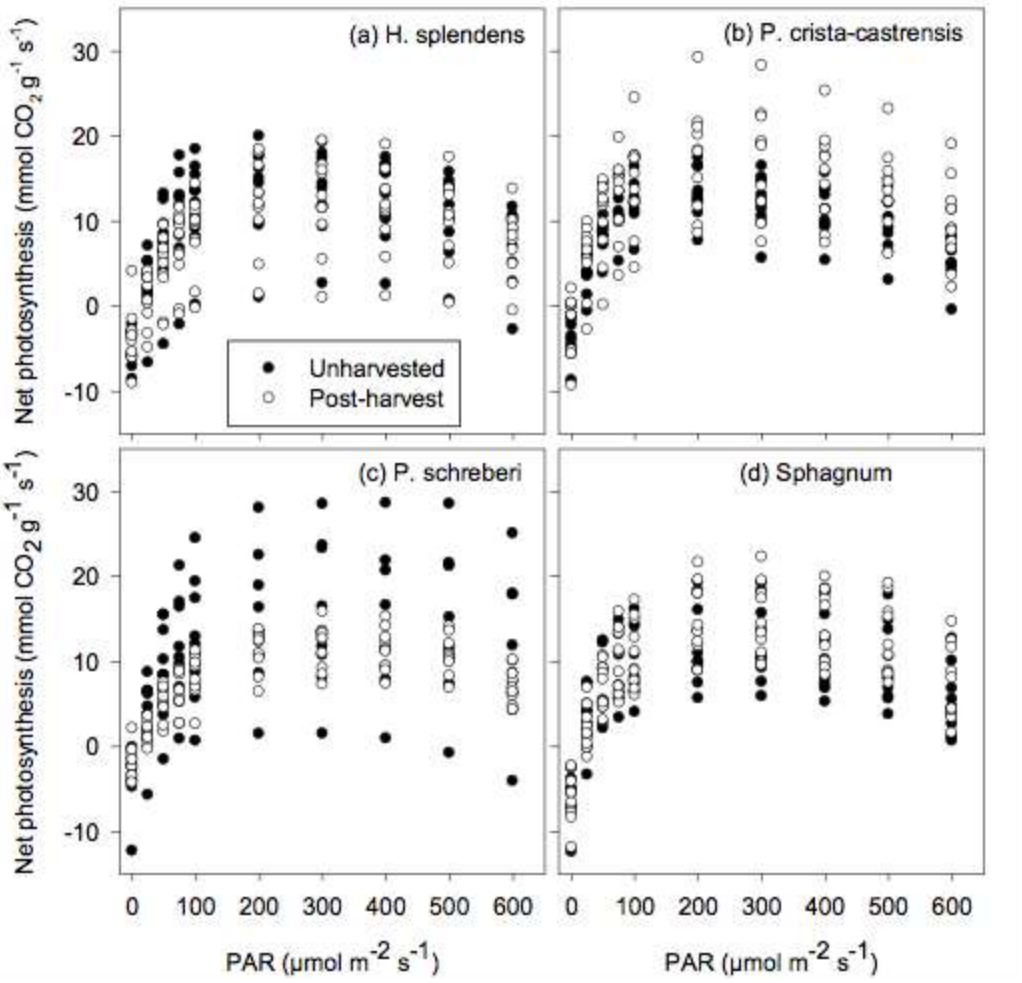

Figure 2-8. Photosynthetic light responses of mosses grown in post-harvest blocks and unharvested blocks in August 2015 for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum.*

2.3.3 Biomass growth

Planted moss stems in unharvested blocks generally had greater monthly increases in biomass for *P. schreberi* and *P. crista-castrensis*, though this trend was less obvious in *H. splendens* due to variations in the early test months (Figure 2-9). The models for *P. schreberi* and *P. crista-castrensis* growth had significant treatment effects ($p<0.0001$), with greater monthly biomass gains in the unharvested blocks, and the sampling month was a significant effect in all three feathermoss species models ($p<0.05$) (Figure 2-9). There was no significant interaction between treatment and month for any of the species. From the middle of June to the end of October, *H. splendens* shoots gained on average 0.0012 g dry weight (8% increase) over the season in the post-harvest blocks, and 0.0063 g (67% increase) in unharvested blocks compared to an average biomass gain of 0.00156 g (25% increase) in post-harvest areas and 0.0021 g (22% increase) in unharvested blocks for *P. crista-castrensis*, and gains of 0.0026 g (36% increase) in post-harvest areas and 0.0048 g (51% increase) in unharvested blocks for *P schreberi* (Figure 2-9). It is noted that *Sphagnum* was not used in the low-density plantation test because of tissue desiccation and death in the field.

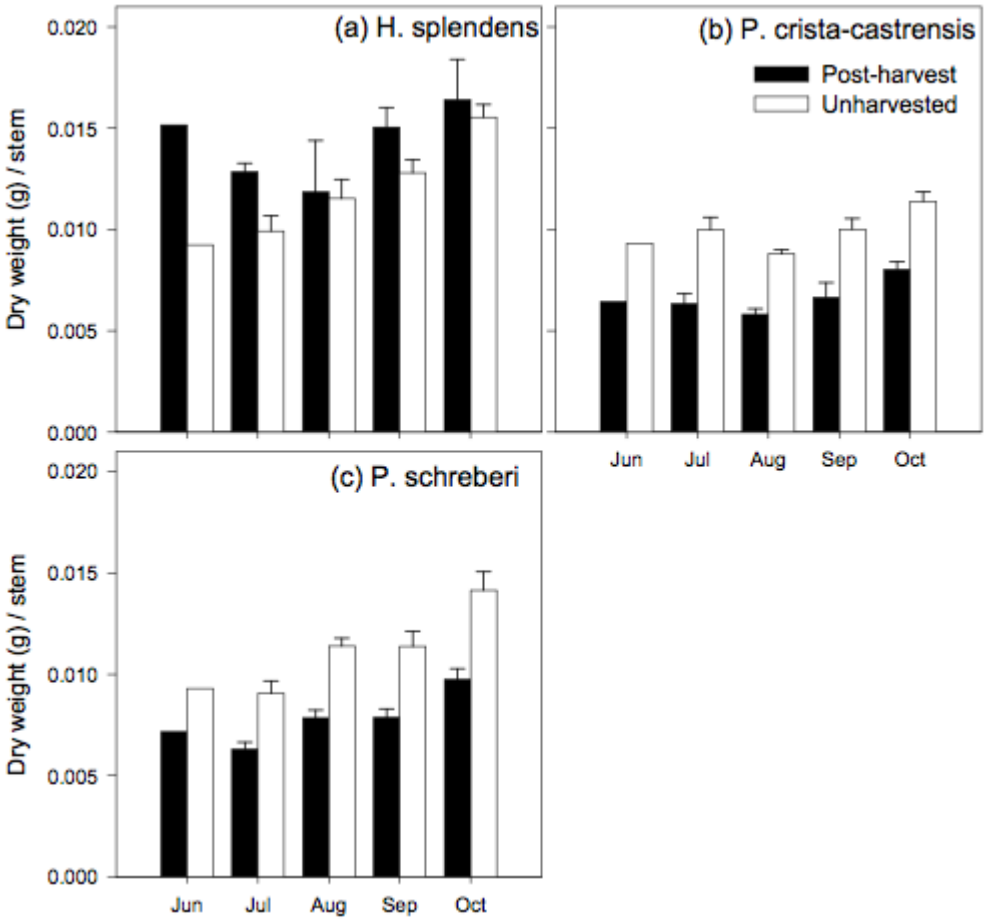

Figure 2-9. Monthly stem weights (with standard error) for feathermosses in both post-harvest blocks and unharvested treatment blocks over the 2015 growing season.

2.3.4 Specific leaf area and shoot density

The greatest stem density for naturally occurring mats was found for *P. crista-castrensis* (20,000 stems/m^2) in the post-harvest blocks, and the lowest natural stem density was *H. splendens* mats in the forest blocks (5200 stems/m^2) (Table 2-5). The increasing stem density of mosses in the post-harvest blocks was significant for *H. splendens* ($p<0.0001$) and *P. crista-castrensis* ($p=0.0242$). The treatment effects were not significant for *Sphagnum* and *P. schreberi* due to high levels of variation among samples.

The specific leaf area (SLA) values found for samples of all species were not significantly different among treatments, though were highly different between all species ($p<0.001$), except for between *P. schreberi* and *H. splendens* (Table 2-5). The ratio was greatest for *P. crista-castrensis*, followed by *P. schreberi*, *H. splendens*, and was lowest for *Sphagnum* (Table 2-5).

Table 2-5. Mean (standard error in parentheses) natural stem density (stems m^{-2}) and specific leaf area (cm^2 g^{-1}) for *H. splendens*, *P. crista-castrensis*, *P. schreberi*, and *Sphagnum* from post-harvest and unharvested blocks.

Species	Treatment	Stem density (stems m^{-2})	Specific leaf area (cm^2 g^{-1})
H. splendens	Post-harvest	11667 (2453)	174.99 (9.01)
	Unharvested	5567 (484)	183.28 (8.64)
P. crista-castrensis	Post-harvest	20000 (2395)	220.29 (9.16)
	Unharvested	12800 (1764)	210.91 (9.67)
P. schreberi	Post-harvest	19933 (2634)	184.40 (8.98)
	Unharvested	17267 (1449)	185.59 (8.96)
Sphagnum	Post-harvest	19467 (2453)	139.60 (6.25)
	Unharvested	16033 (2095)	122.12 (5.44)

2.4 Discussion

2.4.1 Micro-environmental conditions

In the study site, soils were warmer and wetter in the post-harvest areas during the growing season, mainly due to the higher amount of incoming solar radiation and rain which reached to the open areas of ground relative to the floor of the unharvested forest

blocks (Figure 2-2 & 2-3), where the tree canopy can intercept as much as 60% of total rainfall (Price *et al.* 1997). Although relative humidity was not different between the post-harvest area and the forest blocks (data not shown), the higher air temperature significantly increased the vapor pressure deficit (VPD) in the post-harvest areas over the growing season (Figure 2-2). The air temperature was greater in the post-harvest blocks due to the greater irradiance as well, but likely the lower vegetation density in these areas could also have decreased rates of evapotranspiration in these blocks, and therefore more of the incoming energy would be used to heat the air (Carlson *et al.* 2010). The daytime temperature of the post-harvest areas was generally within a reasonable range for boreal moss species, while the unharvested blocks were in the optimal range of 15-25˚C for slightly longer during a given day and more consistently over the growing season (Figure 2-2) (Furness and Grime, 1982). Higher air temperature was seen to exceed 30°C, in the middle of summer only for a few hours at a time (data not shown), and on days of higher irradiance in the post-harvest areas (Figure 2-2).

The proportional ground cover of feathermosses has been known to decrease in the years following clear-cutting or increases in the air temperature of an area, often attributed to higher light levels coupled with greater periods of desiccation (Alatalo *et al.* 2015; Hylander, 2009; Palviainen *et al.* 2005; Press *et al.* 1998), however contrasting responses have also been found (Van Wijk *et al.* 2004). Recovery of moss biomass and relative ground cover after clear-cut harvesting depends both on the regeneration level and the stand age (Bansal *et al.* 2012; Jägerbrand *et al.* 2005). Sufficient soil moisture in both the forest blocks and the post-harvest areas (Figure 2-3 & 2-4) ruled out the possibility of soil water stress affecting the mosses which often occur after tree harvest,

though the effects of sufficient water at depth are questionable as moss shoots are often incapable of drawing water up from depth (Caners *et al.* 2010; Busby and Whitfield, 1978). However, the vapor pressure deficit was consistently higher in the post-harvest blocks than the forest blocks (Figure 2-2), which would mean more frequent and severe shoot desiccation occurred in the post-harvest blocks, which is limiting to biomass growth in mosses (Busby *et al.* 1978; Figure 2-9). Slow growth of mosses in the post-harvest blocks (Figure 2-9) may also be attributed to the high irradiance values measured in the middle summer (Figure 2-2), leading to photo-inhibition (Figure 2-8) as reported by Kubásek *et al.* (2014). In our laboratory tests, for all species in both treatments, photosynthesis reached a saturation point at PAR values of less than 400 µmol m^{-2} s^{-1}, after which any subsequent increases in PAR values led to photo-inhibition and declines in photosynthesis (Figure 2-8). Comparatively, mosses in the unharvested blocks would have received more variable light intensity from shading and bursts of light, which has been shown to increase seasonal growth rates, and overall the light levels found were generally below the point where photo-inhibition occurs (Figure 2-2) (Rincon and Grime, 1989). Besides the potential negative impacts of the high light regime, the open areas of the regenerating stands endured a faster drop in nighttime temperatures relative to unharvested blocks (data not shown), which may have potentially led to damage from repeated freeze-thaw events during the late fall, of which there were a minimum of 2-3 days in October (Bjerke *et al.* 2013; Kennedy, 1993).

2.4.2 Photosynthesis

The overall rates of net photosynthesis for mosses found in this study are within the range reported by others for boreal moss species (Bansal *et al.* 2012). The rates of instantaneous photosynthesis (both net and gross) were greater for all four moss species in the open areas of post-harvest blocks than in the interior of unharvested forest blocks (Table 2-1), but we also found there were consistently higher vapor pressure deficit (VPD) and air temperature in the post-harvest blocks relative to the unharvested forest blocks (Figure 2-2). Higher VDP and air temperature in the post-harvest blocks mean that mosses in such an environment would be more prone to shoot desiccation and thus experience a reduction in time of active photosynthesis over a season. However, our measurements represent a highly active period of photosynthesis as they were carried out only on rainy and/or mist days when natural shoot water content was within an optimal range (Figure 2-5) and the light intensity of the photosynthesis system was set to ambient levels of sampling plots which was almost always lower than the saturation point (~ 400 μmol m^{-2} s^{-1}, Figure 2-8). As PAR levels were much higher in the post-harvest blocks than in the unharvested forest blocks, the greater photosynthesis rates found in samples from these areas are likely due to a positive light response as opposed to being indicative of greater overall plant fitness (Figure 2-8). There was sufficient water in the soil over the growing season due to frequent rain events in this coastal region, and even more so in the post-harvest blocks (Figure 2-3 & 2-4) due to less canopy interception of precipitation, as boreal forest canopies of mature stands can intercept up to 60% of incoming precipitation, limit stem flow, and lower evapotranspiration rates (Price *et al.* 1997). For example, the greater rainfall in the month of June was the likely driver behind

the high levels of net photosynthesis for feathermosses in the beginning weeks of June (Figure 2-6), our results were similar to those of Douma *et al.* (2007) who noted increased importance of moss photosynthesis in the early spring, when moisture can be less limiting (Furness and Grime, 1982). Relatively high rates of instantaneous photosynthesis for the mosses (Figure 2-6 & 2-7) in the post-harvest blocks driven by the increased light (Figure 2-4) may not compensate for the loss of photosynthesis due to dry spells between rain events and photo-inhibition with high irradiance levels often found (Figure 2-8). The lower seasonal biomass growth rates of all feathermoss species in post-harvest blocks relative to those in the unharvested blocks (Figure 2-9) suggest that mosses in the post-harvest areas were subjected to a greater number of metabolically inactive hours, with moisture stress or photo-inhibition as the most likely causes (Huttunen *et al.* 2005).

In comparison to the photosynthetic rates found in the interior forest sampling sites, the higher photosynthesis rates in mosses along the unharvested forest edge blocks (Figure 2-6 & 2-7) suggest that at the time of study, 12 years post-harvest, some mosses are not negatively impacted by their proximity to post-harvest areas (Bansal *et al.* 2012; Nelson and Halpern, 2005). Jonsson *et al.* (2015) measured feathermosses and found higher rates of net photosynthesis for samples grown in smaller patches of intact forest when compared to shoots grown in medium sized forest islands, presumably due to effects much like those found along the forest edge in this study. The greater light transmission to ground cover flora can be utilized to increase productivity, such as with sun-flecks (Kubásek *et al.* 2014) and the overall impact of light on photosynthesis (Figure 2-8), while moisture retention under the tree canopy is presumed to be somewhat retained. The higher light levels on the edges of the forest area were comparable to a late-

successional boreal stand in which light is less of a limiting factor, and this has been shown to positively impact moss NPP (Jonsson *et al.* 2015). It should be noted, however, that edges of a different orientation or slope may experience much greater changes in microclimate conditions and therefore alternate effects on species' productivity, with wind speeds or sunlight changing more frequently, and therefore the results found here may not be applicable in other areas (Nelson and Halpern, 2005).

The high photosynthesis rates observed in mosses of the unharvested forest blocks over the first two test days at the beginning of the growing season(Figure 2-6 & 2-7) are likely due to high moisture levels from rainfall, fewer daylight hours, and lower air temperatures (Figure 2-2), allowing a peak in productivity (Furness and Grime, 1982). This did not continue over the growing season, as overall low instantaneous photosynthesis rates found in the interior of the unharvested forest areas are likely due to the extremely low PAR measurements under the canopy on the rainy and misty measurement days (Figure 2-4). However, over a growing season the mosses from the forest interior areas were likely able to greatly exceed the total number of photosynthetically active hours experienced by mosses in the post-harvest blocks, because the shoots from the unharvested blocks were less vulnerable to shoot desiccation under relatively-low VPD (Figure 2-2). The high growth rates enabled by the more amenable moisture conditions were likely also a result of sun-flecks thought to be common in black spruce forests. The compact foliar growth form and narrow canopy of black spruce trees leave large gaps through which intense bursts of sun are common, and these sun-flecks are thought to facilitate the high productivity of ground cover species in

the forest interior (Swanson and Flanagan, 2001; Tobias and Niinemets, 2010; Botting and Fredeen, 2006).

The differences in treatment effects among species are likely due to differences in morphological and growth form characteristics. A study by Huttunen *et al.* (2005) found different average specific surface areas ($cm^2\ g^{-1}$) for a variety of boreal moss species such as was found in the present study, meaning that for similar masses they had different amounts of leaf area which could capture light to use for photosynthesis, potentially different ratios of photosynthetic to water holding cells, and different relative surface areas through which water could evaporate. These differences in SLA can impact water retention for individual shoots, and can lead to the different density responses of species, as the shoot shape and volume alter the inter and intra-shoot shading within the mat, as well as the humidity and moisture conditions within the active upper mat layer (Table 2-5) (Pederson *et al.* 2001). Differences in shoot density per unit area, such as was found among treatments for species (Table 2-5), would result in different levels of competition for resources; Pederson *et al.* (2001) found that shoots in high density colonies (the post-harvest blocks) experience increased competition for light resources, and that this negatively impacted growth rates and offshoot number of branches. Some of the moss species that displayed less of a treatment effect (*P. crista-castrensis* and *Sphagnum*) may also have acclimatized to the higher temperatures a decade following a clear-cut harvest; Wagner *et al.* (2012) found a positive correlation between temperature responses in bryophytes and ambient temperatures in their environment, and also suggested that bryophytes could adapt to changes in mean temperatures.

The negative effects of tree harvest on *H. splendens* compared to other feathermosses in this study (Figure 2-6 & 2-7) have been previously noted, and the productivity of *H. splendens* over a season has been correlated with total precipitation and moisture stress (Caners *et al.* 2010). *H. splendens* has previously been found to have growth rates that drop off sharply with temperatures above its optimum with a stronger negative response than other feathermosses (Furness and Grime, 1982). It was surprising that its net photosynthesis rates on rainy days were often comparable to those of other feathermoss species, as it had been found previously to have lower NPP values than *P. schreberi* (Jonsson *et al.* 2015). Shoots of *H. splendens* were at a greater risk of desiccation in the post-harvest blocks, due to their weaker water retention abilities (Figure 3-4), and the growth form which spreads much more laterally than the other species (personal observation), leading to greater relative surface area which is fully exposed to sunlight in natural conditions. The nearly four-fold increase in shoot density for moss mats in the regenerating areas was much greater than for any other species (Table 2-5), which may indicate a stronger response to moisture stress or light-avoidance.

Comparatively, *P. schreberi* has been found to be very successful in becoming a dominant species in newly formed forest gaps and has been reported to recover faster than other species after clear-cutting due to highly efficient spreading when conditions are favorable and a drought tolerant nature (Frego, 1996; Schmalholz and Hylander, 2009). In the present study it was found to have mid-range net and gross photosynthesis rates as well as SLA ratios when compared to other study species, with high shoot densities found in both regenerating and unharvested forest areas (Table 2-5). These features of *P. schreberi* support the current findings, which suggest a lack of a treatment effect on

photosynthesis rates, and a study by Bansal *et al.* (2012) also found no strong relationship between gross photosynthesis and light transmission in a boreal environment for this species. Additionally, *P. schreberi* growth rates in natural turfs have been shown to be less affected by increased environmental temperature than other feathermosses (Furness and Grime, 1982), a phenomenon which could have aided shoots in the post-harvest blocks. However, the differences in biomass gains in the low density growth plates in regenerating stands suggest that moisture stress can strongly limit biomass gains over a season (Figure 2-9).

P. crista-castrensis has numerous small branches and a sub-erect growth form which can facilitate water retention, but have been found to lead to relatively higher rates of self-shading within moss mats regardless of shoot density (Pederson *et al.* 2001). The high rates of photosynthesis found in the present study contrast to the generally low growth rates over a range of environments found by others and the present study (Pederson *et al.* 2001; Proctor 1990), though this could be explained partially by the SLA ratios found, as photosynthesis rates are calculated in terms of dry weight, a gram of *P. crista-castrensis* would have on average 15-40% more surface area over which light can be absorbed (Table 2-5). Others often report findings based on ground area as opposed to dry weight, and the low stem density found in the unharvested blocks could lead to more similar results.

The greater water holding capacity of *Sphagnum* is a characteristic feature of this family, and enables shoots to better retain water in high light environments, especially when compared to feathermosses (Figure 3-4) (Sveinbjornsson and Oechel, 1992; Bisbee *et al.* 2001; Swanson and Flanagan, 2001). The presence of *Sphagnum* in an area can lead

to changes in the soils below these mats, which are often reported to waterlog soils and decrease pH levels as they draw water up from depth (Bates and Farmer, 1992; Bisbee *et al.* 2001). *Sphagnum* mats most commonly grew in wetter areas of the black spruce forests with relatively low tree density, and the *Sphagnum* shoots maintain these moist conditions through denser growth patterns and high shoot water holding capacity (Table 2-5 & Figure 3-4) (Bisbee *et al.* 2001). This was true in the open areas of the post-harvest blocks as well, where *Sphagnum* was able to remain metabolically active for longer due to the waterlogged soils and shoot water retention abilities, and could potentially have photosynthesized at the higher rates found for a similar amount of time as in unharvested forest blocks. *Sphagnum* presence can often be positively correlated with PAR values in an area due to canopy opening, while the reverse was true for feathermosses, supporting the present study findings that *Sphagnum* shoots are highly productive in exposed areas with little canopy cover (Table 2-1 & Figure 2-6) (Bisbee *et al.* 2001; Hylander, 2009).

2.5 Conclusion

Although the instantaneous rates of both gross photosynthesis and net photosynthesis of mosses were found to be higher for the post-harvest areas relative to the unharvested controls, its lower growth rates indicate the increased vapor pressure deficit may cause pronounced levels of moisture stress for mosses in harvested blocks. The ability of moss grown in the unharvested areas to better retain shoot moisture can reduce the risk of moisture stress, and over a growing season can allow shoots to remain metabolically active for much longer, thereby increasing their growth rates compared to shoots in harvested blocks. Such differences could potentially impact productivity for

years to come, as some studies suggest that boreal black spruce forests regrow in a patchier manner, with some never achieving a closed canopy (Dussart and Payette, 2002).

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Chapter 3: Contrasting photosynthetic light response parameters and pigment contents of boreal mosses in harvested and unharvested black spruce stands

3.1 Introduction

Globally, the boreal forest is thought to contain up to 40% of the terrestrial carbon (C) pool, and it is a vital economic resource (Apps *et al.* 1993; Gower *et al.* 2001). Boreal forests are considered a net C sink, annually increasing stored pools of C in small increments often attributed in largely to the high levels of annual productivity of bryophytes on the forest floor (Goulden and Crill, 1997). Concerns are growing over potential shifts in the net C balance of forests in the years following harvesting, and more information is needed on the large and small scale harvest effects on ground bryophytes. Harvesting of trees can increase the air temperature and wind speed in an area, while decreasing moisture available to plants (Arsenault *et al.* 2012); this can be problematic for mosses common to boreal forest floors which are widely considered to be shade plants, doing best in low light or high moisture environments (Benscoter and Vitt, 2007; Marschall and Proctor, 2004; Proctor 1990). A near continuous understory cover of bryophytes is a characteristic feature of boreal black spruce forests, with *P. schreberi*, *H. splendens*, and *P. crista-castrensis* most often present (Benscoter and Vitt, 2007; Bisbee *et al.* 2001).

Mosses are found over a wide range of habitats, but in most areas moisture is a limiting factor to productivity (Marschall and Proctor, 2004). Mosses are small

poikilohydric shoots with no true root system, reliant on external moisture sources to regulate their water content, and they lack the stomata used by vascular plants to limit water loss (Dilks and Proctor, 1979; Marschall and Proctor, 2004). It is advantageous for mosses to exist in low light environments, as high light conditions increase evaporation of water, potentially rendering mosses photosynthetically inactive, and can also decrease photosynthetic rates of mosses due to photo-inhibition (Kubásek *et al.* 2014; Müller *et al.* 2016; Proctor and Tuba, 2002). Mosses are able to thrive in low-light environments because their photosynthesis rates often reach maximum levels at low light intensities, with saturating light levels equal to 20% of full sunlight found for boreal mosses, and saturating light levels in other environments are also often linked to the average daily radiation of an area (Kershaw and Weber, 1986; Lappalianen *et al.* 2008; Marschall and Proctor, 2004; Bergeron *et al.* 2009).

Mosses can adapt to changing microclimate conditions by shifting physiological and functional shoot traits, often altering photosynthetic responses to varying light levels and photosynthetic pigment concentrations (Hoddinott and Bain, 1979; Lichtenthaler *et al.* 2013). Photosynthetic responses to altered microclimate regimes can vary between species due to morphological and functional trait differences, with surface area and growth forms differences often as the key factors (Arsenault *et al.* 2012; Waite and Sack, 2010). Light response curves are often used to evaluate photosynthetic responses to continuously changing variables such as light (Peek *et al.* 2002), and light response curves are commonly used to examine the changes in photosynthetic rates with changing irradiance (Rice *et al.* 2008). Under low light conditions plants are limited by the rate of electron transport, and in high light conditions by Rubisco capacity, which leads to a light

response curve that can be accurately fitted by the non-rectangular hyperbola model of Farquhar *et al.* (1980). Parameterized light response curves can be used to determine maximum photosynthesis rate (P_{max}), respiration rate (R_D), quantum efficiency (ε), the initial rate of change at low light levels, the saturating light level (L_{Sat}), and the light compensation point (L_{CP}) - the light level at which the net carbon flux is 0 (Mbufong *et al.* 2014; Rice *et al.* 2008). Generally, plants growing in high light environments have greater photosynthetic capacities, light compensation levels, and saturating irradiance levels when compared to plants grown in light limited environments (Ueno *et al.* 2006). It is thought that light responses in plants aid in optimizing the amount of light which can be intercepted by the plant, increasing potential rates of photosynthesis (Lichtenthaler *et al.* 2013).

In addition to alterations of photosynthetic response parameters, plants can also respond to altered habitat conditions by adjusting photosynthetic pigment concentrations. Chlorophylls and carotenoids, the most common and abundant pigments within terrestrial plants, are integral to photosynthesis and photo-protection (Czeczuga, 1987; Fu *et al.* 2012; Wrolstad *et al.* 2005). Chlorophyll and carotenoid content often increase with light availability (Jägerbrand *et al.* 2005; Jägerbrand *et al.* 2012) and changes in concentration of these pigments has been used to assess photosynthetic abilities and the relative partitioning of energy between photosynthetic and structural tissues (Rice, 1995).

Changes in chlorophyll concentration in plant leaves have been seen in response to abiotic or biotic stress, and are often studied in reference to changes in irradiance (Dale and Causton, 1992; Hu *et al.* 2013). Chlorophylls are actively involved in photosynthetic light harvesting, energy transduction, and are present within the reaction centers (Fu *et al.*

2012; McCall and Martin, 1991). Chlorophyll a (Chl *a*) is found in reaction centers of photosystems I and II, as well as within the pigment antenna, while chlorophyll b (Chl *b*) is found only in the pigment antenna (Lichtenthaler and Buschmann, 2001). The light harvesting center of photosystem I has a relatively constant ratio of Chl *a*:*b*, while in photosystem II this ratio can change in response to light levels, and it is the changes in photosystem II which are detected during analysis (Lichtenthaler and Buschmann, 2001; McCall and Martin, 1991). Increases in Chl *b* concentrations relative to Chl *a* may allow plants on the light limited forest floor to capture more photons, as tree canopies can often absorb more light in the Chl *a* absorption band (Boardman, 1977). Mosses are often reported to have Chl *a*:*b* ratios from 1.5-3, much lower than commonly found in vascular plants, and another supporting argument for the classification of mosses as shade plants (Marschall and Proctor, 2004; Martin and Churchill, 1982). Lichtenthaler *et al.* (2013) found that leaves which grow in high light environments have greater maximum photosynthetic rates and less total chlorophyll content on a dry mass basis when compared to leaves grown in low irradiance conditions. For shade grown plants, the light limiting nature of their environment prompts the funneling of resources into the production of Chl *a* and *b* proteins so that light can be utilized.

Carotenoids are thought to help plants use a maximum amount of incoming irradiance in low-light situations, while also potentially protecting chlorophyll pigments from photo-destruction in high irradiance situations (Czeczuga, 1987; Fu *et al.* 2012). Mosses have been reported to increase carotenoid concentration with increasing habitat irradiance levels, presumably due to higher photo-protection needs of shoots (Rice *et al.* 2008). The ratio of total chlorophylls to carotenoids is often greater in shade grown

plants, and can be used as a means to compare the "greenness" of a plant sample; in situations of stress, damage, or senescence, chlorophyll breaks down faster than carotenoids, which lowers the ratio (Lichtenthaler and Buschmann, 2001). Plant material grown under high light conditions were also found to have lower Chl *a*:*b* ratios, and lower chlorophyll: carotenoid ratios than leaves grown in the shade (Lichtenthaler *et al.* 2013).

The current study sought to assess the variety and degree of light adaptations found in feathermosses and *Sphagnum* in previously clear-cut areas of a black spruce forest over a growing season, as compared to those same species in the unharvested forests nearby. It was hypothesized that clear-cut/post-harvest areas would have a negative effect on total chlorophyll concentrations but would lead to greater maximum photosynthetic rates, as light would no longer be a limiting resource in the more open environment. Additionally, the ratio of Chl *a:b* would be greater in more open areas, again as less energy needs to be expended to maintain photosynthetic levels, while the ratio of chlorophylls: carotenoids would be lower as plants browned and photo-protection needs increase. The response of all feathermoss species were assumed to be similar, while it was hypothesized that *Sphagnum* may exhibit a response more closely related to those seen in vascular plants due to their occurrence in high light environments in many other habitats.

3.2 Methods

3.2.1 Site Description

The study site used in Chapter 2 was also used for this set of experiments. Sample areas were spread across all eight blocks (four post-harvest and four unharvested) such

that each block had 2-3 sampling microplots within it that were used consistently over the growing season, for a total of 10 microplots for the post-harvest treatment and another 10 for the unharvested treatment, and from each microplot samples of *H. splendens*, *P. crista-castrensis*, *P. schreberi*, and *Sphagnum* were collected. Collections were made within 24 hours of a rainfall event during the last week of every month from June-November 2015.

3.2.2 Light response curves

Moss samples were collected for light response curves from 10 microplots within each treatment (post-harvest and unharvested, the same as mentioned in 3.2.1) at the end of each month from June-November 2015. Samples were left to equilibrate for 72 hours in a Conviron Doirma plant growth chamber (daytime: 12 hours at 14 ^{o}C, >75% RH, ~100 µmol m^{-2} s^{-1} light intensity; night: 12 hours at 12 ^{o}C, >75% RH, complete darkness) prior to measurements. Shoots were removed from the growth chamber an hour prior to testing and hydrated to full turgidity. Each sample consisted of four moss shoots of a given species cut in the same manner as samples in Chapter 2. Samples were gently blotted on a kimwipe before testing, and placed horizontally without leaf overlaps on the raised mesh of the Li-6400XT portable photosynthesis system equipped with LI-6400-24 Bryophyte Chamber (Li-Cor Biosciences, Lincoln, NE, USA). During the measurements, the chamber fan was set to medium, and all environmental parameters in chamber remained constant (relative humidity 80%; air temperature 20 ^{o}C; CO_2 concentration 390 ppm) except PAR, which was programmed to decrease sequentially from strong to weak (600, 500, 400, 300, 200, 100, 75, 50, 25, and 0 µmol m^{-2} s^{-1}) (Wang *et al.* 2016; McCall

and Martin, 1991). Samples were given two minutes to be acclimatized to each light level before net CO_2 exchange readings were made, and the dark respiration rate was determined when PAR was 0 μmol m^{-2} s^{-1}. Each sample curve took 20 minutes to complete; this was determined to be a short enough time-span that impacts of drying on CO_2 fluxes would be minimal based on visual examination of drying curves described in the following section. Shoot leaf area was measured using Li-3300C (Li-Cor Biosciences, Lincoln, NE, USA), and specific leaf area was calculated as the ratio of leaf area to dry weight. Samples were dried for 48 hours at 75 ˚C and then weighed. Once completed, CO_2 exchange rates were recalculated to be expressed as CO_2 fluxes per g dry weight (Wang *et al.* 2016).

Individual response curves were fit with the non-rectangular hyperbolic function (Farquhar and Wong, 1984):

$$\theta(A + R_d) - (\varepsilon Q + P_{max})(P_{max} + R_d) + \varepsilon Q P_{max} = 0 \quad (1)$$

where A is the net photosynthesis rate (mmol CO_2 g^{-1} s^{-1}), Q is the incoming radiation (μmol m^{-2} s^{-1}), P_{max} is the maximum gross photosynthesis rate at light saturating point (mmol CO_2 $g^{-1}s^{-1}$) , ε, the initial curve-slope, is the apparent quantum efficiency, θ is a measure of convexity of the response curve, and R_d is the rate of respiration measured at PAR=0 μmol m^{-2} s^{-1} (mmol CO_2 $g^{-1}s^{-1}$) (Whitehead and Gower, 2001). The light compensation point (Lcp) is the incoming radiation when A was set to 0 and the other fitting coefficients from curve fitting analysis were held as constants (Kubásek *et al.* 2014). The light saturation at 95% ($95\%L_{Sat}$) is the incoming radiation needed to obtain 95% of the rate of P_{max} (Waite and Sack, 2010).

3.2.3 Drying curves

Samples of all four test species were collected from microplots within the eight blocks to establish a full set of drying curves. Samples were left in the same growth chamber as previously mentioned for 24-48 hours of equilibration prior to measurements. Samples were hydrated for one hour to full turgidity in the laboratory prior to testing, and the top 2 cm of green intact stem was used for testing, with the top leaf segment used for *H. splendens*. Initial saturated weights were recorded after gently shaking off excess water, and a measurement program was run on the Li-6400XT (two minutes of light and then two minutes of darkness), PAR was set at 500 µmol m^{-2} s^{-1} during lighted test portions for all net photosynthesis measurements, as this was presumed to be an irradiance above light saturation for all species tested (Rice *et al.* 2011), and other variables were consistent with those used for the light response curves. Gross photosynthesis was calculated as the sum of the net photosynthesis and respiration (Davey and Rothery, 1996). Samples were removed from the bryophyte chamber and left for 30 minutes on the laboratory bench. New weights were recorded after 30 minutes and the samples were re-measured in the Li6400XT. This process continued until net photosynthesis rates and respiration rates were essentially zero (Rice *et al.* 2011).

3.2.4 Pigment Analysis

Samples for pigment analysis were collected on a monthly basis on the same day and from the same microplots as the light response curves samples, with 10 microplots used for the post-harvest and the unharvested treatments. Shoots were given 24 hours to

equilibrate in the growth chamber, and the top 2 cm of each shoot was separated and frozen at -20 ˚C until analysis in January 2016, with the exception of *H. splendens* which was cut just below the upper leaf segment. Once removed from the freezer, all subsequent steps were performed in very dim lighting. Samples were homogenized in a Cryomill (Retsch, Germany) with a 1cm diameter ball bearing for 30 seconds at 30 Hz. Of the ground material, three 50 mg samples were weighed, transferred to 2 mL micro-cuvettes, and placed again in the -20 °C freezer, while two 50 mg samples were dried for 48 hours at 75 ºC and weighed to obtain dry weight (Jägerbrand *et al.* 2005). The dry to fresh weight ratio was determined, and the average of the two ratios was used to express chlorophyll concentrations per gram of dry weight. Samples were removed from the freezer and prepared by adding 1.5 mL of 80% aqueous (v/v) acetone to each micro-cuvette, and cuvettes were then centrifuged for 10 minutes at 3000 G (Jägerbrand *et al.* 2005). 200 µmol of the supernatant was pipetted into each microplate well for absorbance reading using a Biotek Synergy HT microplate reader (Winooski, VT, USA). Absorbance was recorded at 470, 647, 663, and 750 nm (Jägerbrand *et al.* 2005; Lichtenthaler and Buschmann, 2001). Pigment concentrations were determined using Lichtenthaler and Buschmann's equations (2001) for 80% acetone:

$$c_a\ (\mu g ml^{-1}) = 12.25A_{663.2} - 2.79A_{646.8} \quad (2)$$

$$c_b\ (\mu g ml^{-1}) = 21.50A_{646.8} - 2.79A_{663.2} \quad (3)$$

$$c_{(x+c)}\ (\mu g ml^{-1}) = (1000A_{470} - 1.82c_a - 85.02\ c_b)/198 \quad (4)$$

C_a is the concentration of Chl *a*, C_b is the concentration of Chl *b*, $C_{(x+c)}$ is the

concentration of total carotenoids, and A is the absorbance at a wavelength denoted in subscript. In order to adjust microplate absorbance values to be used in the above equations, a path-length correction of 1.73 was used based on the methods of Warren (2008), and the correction was found by creating a Chl *a* standard curve (2-10 µg mL^{-1}) on the microplate reader prior to sample testing. The path length was determined to be 0.58 cm.

3.2.5 Statistical Analysis

The light response curve fitting was conducted using Sigma Plot 11. The effects of treatment on photosynthetic parameters and pigment contents were analyzed using linear mixed models based on the randomized experiment design with repeated measures over time. The mixed model involved two factors: treatment (post-harvest and unharvested) and sampling month (June-November). The model contained the fixed effects of treatment, month, and their interaction, the random effect of blocks nested in the treatment, and a statement to account for the repeated nature of the experiment. Least square difference was used to test for differences in photosynthetic parameters and pigment contents among species in clear-cut and control treatments, and to test for differences in mat densities, optimal water contents, and measures of specific leaf area ($p<0.05$). The type I error threshold was fixed at $\alpha=0.05$. All mixed model analyses were performed using SAS version 9.3.

3.3 Results

3.3.1 Photosynthetic parameters

Quantum efficiency

The rate of apparent quantum efficiency (ε) ranged from roughly 0.1-0.4 over the season, and generally was greater in samples from the unharvested blocks than from the post-harvest blocks, regardless of moss species, with rates in both treatments peaking in August and September (Table 3-1 & Figure 3-1). Monthly rates were significantly lower in the post-harvest blocks than in the unharvested blocks for *P. crista-castrensis* in July ($p=0.0165$), *P. schreberi* in July ($p=0.0498$) and August ($p=0.0004$), but remarkably higher in *Sphagnum* from the post-harvest sites in June ($p=0.0126$) (Figure 3-1). Though monthly means appeared different between treatments for *H. splendens*, no significant treatment effect over any one month was found (Figure 3-1). Within the samples from the post-harvest blocks, ε was significantly greater for *Sphagnum* (*H. splendens*, $p=0.0014$; *P. crista-castrensis*, $p=0.0024$; *P. schreberi*, $p<0.0001$), followed by *P. crista-castrensis* and *H. splendens* which were not different from each other but both had significantly greater rates than *P. schreberi* (*H. splendens*, $p=0.0125$; *P. crista-castrensis*, $p=0.0092$) (Table 3-1). In the unharvested blocks ε rates were significantly lower for *H. splendens* (*P. crista-castrensis*, $p=0.0352$; *Sphagnum*, $p=0.0115$), and no other significant differences were found among species (Table 3-1). A treatment effect on ε was found for *P. schreberi* and *P. crista-castrensis*, but not for *H. splendens* or *Sphagnum* (Table 3-2). The sampling month had a significant effect on ε for all species ($p<0.001$), and there was a significant interaction between sampling month and treatment for *Sphagnum* ($p=0.0356$) (Table 3-2).

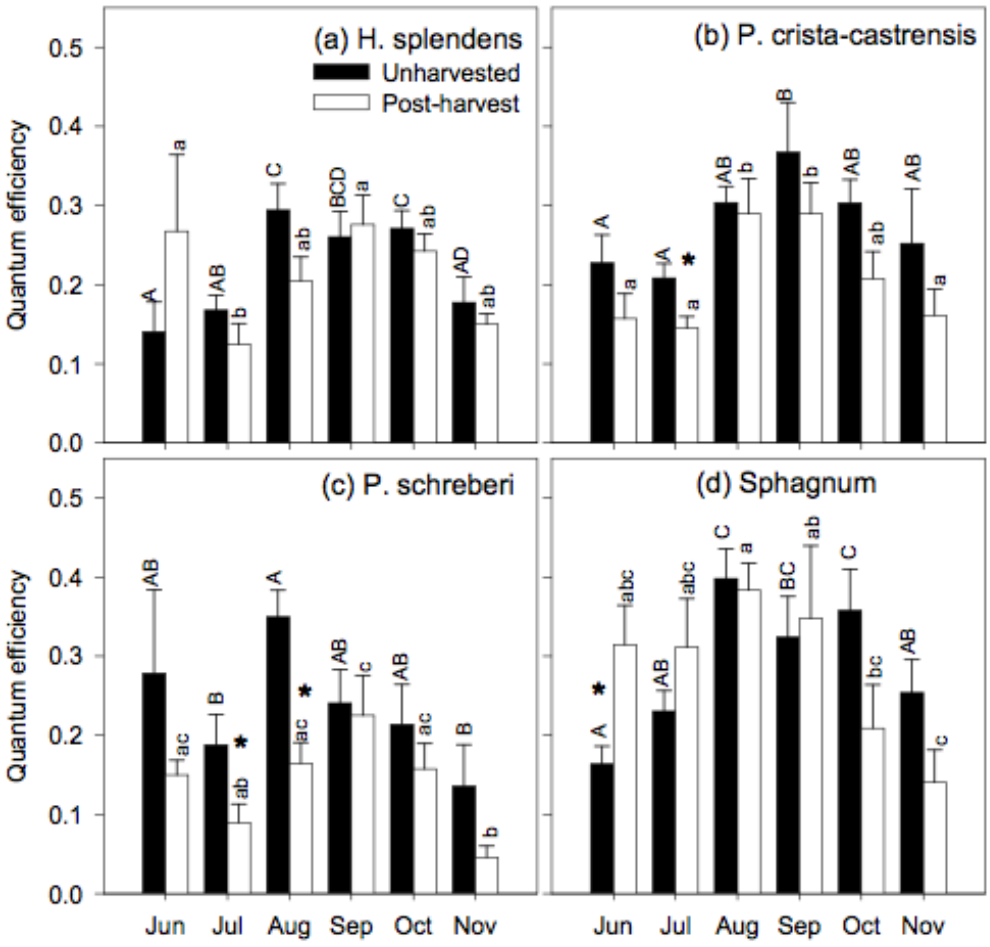

Figure 3-1. Mean value (with standard error) of quantum efficiency (ε) rates from June-November 2015. Values are averages of 10 individually fit curves for both the post-harvest and the unharvested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=10). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Maximum photosynthesis

Maximum photosynthesis (P_{max}) generally stayed within the range of 5-20 mmolCO^2 g^{-1} s^{-1} over the growing season, with peaks in August and September and a decline in November (Figure 3-2). P_{max} was significantly greater for *P. schreberi* from the unharvested blocks than from the post-harvest blocks in July ($p=0.0429$) and August ($p=0.0169$), while greater P_{max} was found for *Sphagnum* from the post-harvest blocks during June ($p=0.0047$) and July ($p=0.0351$) (Figure 3-2). No differences were found in any one month between the unharvested and post-harvest block for *H. splendens* or *P. crista-castrensis* (Figure 32). Among species sampled from the post-harvest blocks, P_{max} was larger for *Sphagnum* and *H. splendens* than *P. schreberi* (*Sphagnum*, $p<0.0001$; *H. splendens*, $p=0.0004$) and *P. crista-castrensis* (*Sphagnum*, $p=0.0093$; *H. splendens*, $p=0.0246$) (Table 3-1). In the unharvested blocks there were no seasonal differences in Pmax among species (Table 3-1). Seasonal Pmax was higher for Sphagnum from the post-harvest blocks than from the unharvested blocks ($p=0.0327$), and was greater for P. schreberi from the unharvested blocks as opposed to the post-harvest blocks ($p=0.0427$) (Table 3-1 & Figure 3-2). Mixed model results showed significant effects of sampling month on P_{max} for all species, but no treatment effects and/or significant interactions between treatment and sampling month were detected (Table 3-2).

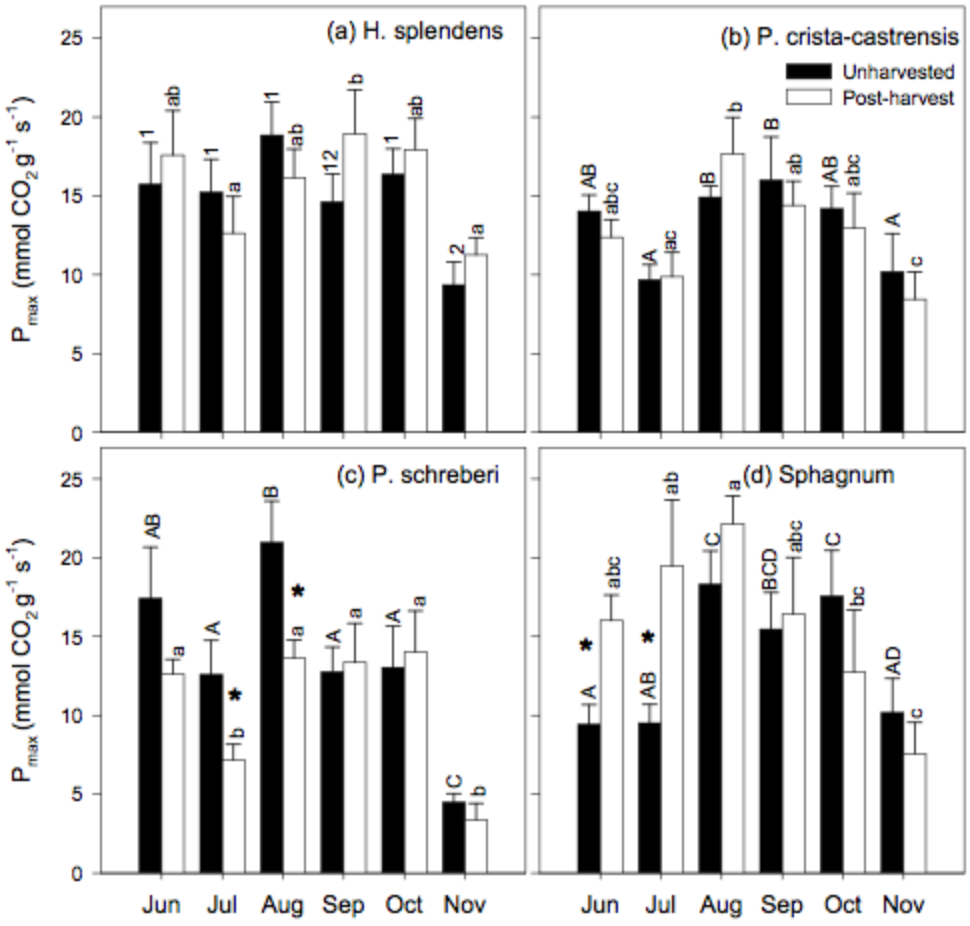

Figure 3-2. Mean rates (with standard error) of maximum gross photosynthesis (P_{max}, mmol CO_2 g^{-1} s^{-1}) rates from June-November 2015. Values are averages of 10 individually fit curves for both the post-harvest and the unharvested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=10). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Light compensation point

Light compensation levels were highly variable, ranging from 40-150 µmol m^{-2} s^{-1} (Table 3- &, Figure 3-3).A general trend of decreasing values towards the middle test months and increasing levels as fall progressed were seen (Figure 3-3). Light compensation points for *P. crista-castrensis* and *H. splendens* tended to be greater in samples from the post-harvest blocks, while *Sphagnum* had a high variability, and *P. schreberi* levels were similar between the treatments (Figure 3-3). No significant differences were found among seasonal means of species within each treatment (Table 3-1). Mixed model results had no significant effects of treatment or sampling month and treatment interaction, but the sampling month was a significant effect for *H. splendens, P. schreberi*, and *Sphagnum* (Table 3-2).

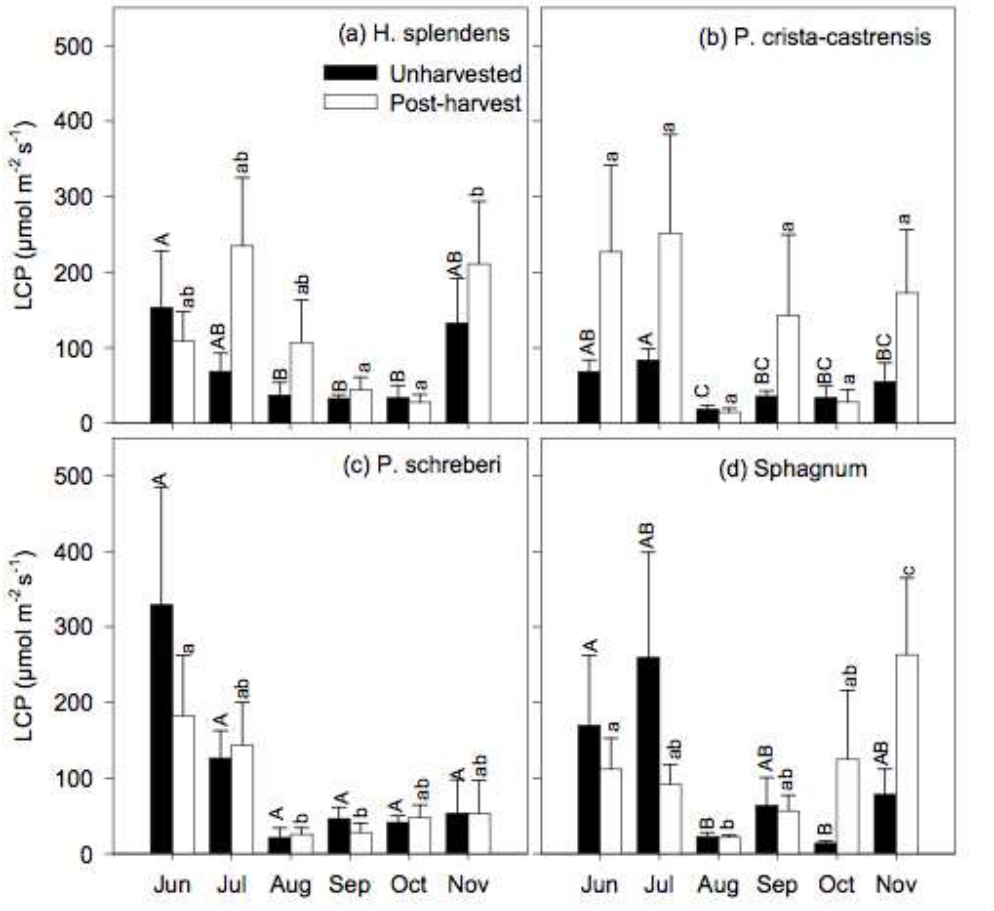

Figure 3-3. Monthly mean values (with standard error) of the light compensation point (Lcp, µmol m^{-2} s^{-1}) rates from June-November 2015. Values are averages of 10 individually fit curves for both the post-harvest and the unharvested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=10). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

95% light saturation

Light levels needed to reach 95% of the maximum photosynthesis rates ($95\%L_{Sat}$) were almost always higher in the samples from the post-harvest blocks regardless of species, ranging from: 53-132 µmol m^{-2} s^{-1} for *P. crista-castrensis*, 73-333 µmol m^{-2} s^{-1} for *P. schreberi*, 182-388 µmol m^{-2} s^{-1} for *H. splendens*, and 78-367 µmol m^{-2} s^{-1} for *Sphagnum* (Table 3-1 & Figure 3-4). No seasonal trend was apparent in $95\%L_{Sat}$ values for samples of *H. splendens* and *Sphagnum* from the post-harvest blocks, while *P. crista-castrensis* and *P. schreberi* had steadily decreasing $95\%L_{Sat}$ values in samples from the unharvested blocks (Figure 3-4). In the post-harvest blocks, the values of $95\%L_{Sat}$ were greater for *H. splendens* than for *P. crista-castrensis* ($p<0.0001$) and *P. schreberi* ($p=0.0250$), and values for *Sphagnum* and *P. schreberi* were greater than for *P. crista-castrensis* (*Sphagnum*, $p<0.0001$; *P. schreberi*, $p=0.0012$) (Table 3-1 & Figure 3-4). Moss samples from the forested blocks generally had decreasing $95\%L_{Sat}$ values as the growing season progressed, $95\%L_{Sat}$ levels for *P. schreberi* and *Sphagnum* in November were half as large as in June (Figure 3-4). Within the samples from the forest block, $95\%L_{Sat}$ values for *P. crista-castrensis* were lower than those of all other species (*H. splendens*, $p= 0.0174$; *P. schreberi*, $p=0.0216$; *Sphagnum*, $p=0.0272$), and no other differences among species were significant (Table 3-1 & Figure 3-4). Models detected a significant effect for sampling time for *P. crista-castrensis* and *P. schreberi* though the treatment effect was only significant in *H. splendens* which had higher values in samples from the post-harvest block, and there was no significant interaction between treatment and sampling month (Table 3-2).

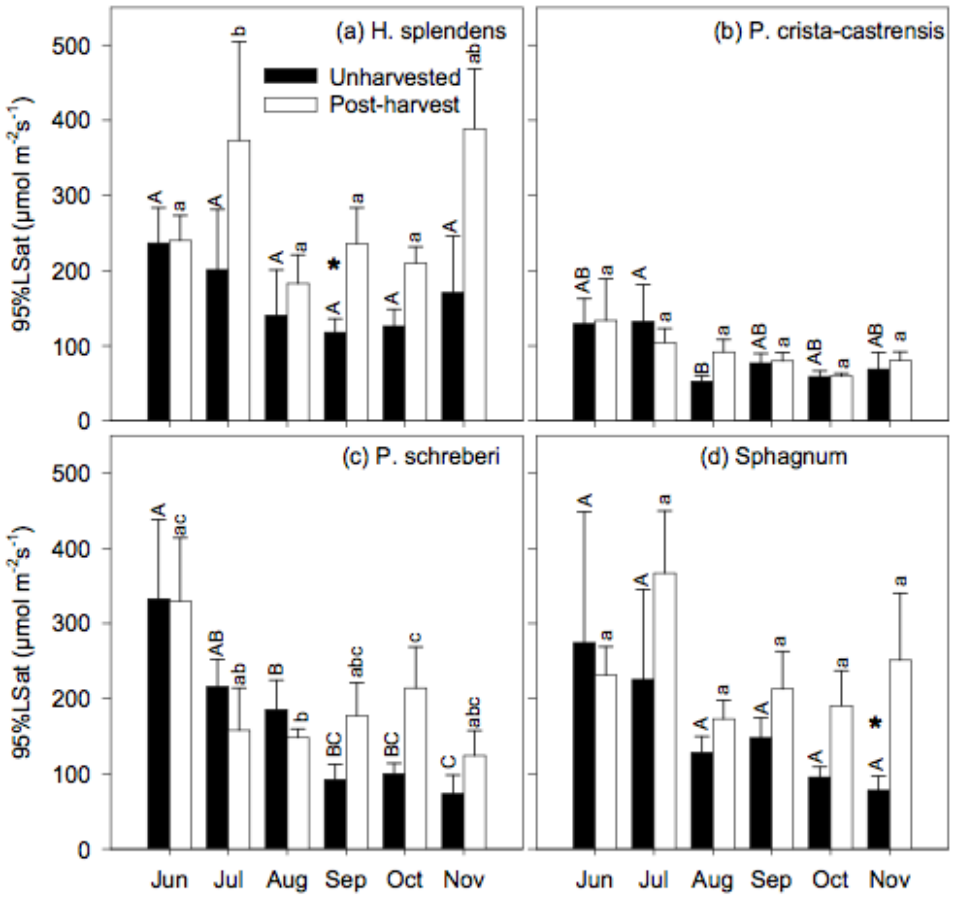

Figure 3-4. Monthly mean values (with standard error) of the light intensity needed to reach 95% of the maximum photosynthesis rate (95%L_{Sat}, µmol m^{-2} s^{-1}) from June-November 2015. Values are averages of 10 individually fit curves for both the post-harvest and the forested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=10). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Table 3-1. Seasonal means (with standard error in parentheses) of light response curve parameters for *P. crista-castrensis*, *P. schreberi*, *H. splendens*, and *Sphagnum* collected in post-harvest and unharvested forest blocks over the 2015 growing season (June – November). Uppercase letters represent differences in values among species within the post-harvest blocks; lowercase letters denote differences among species within the unharvested blocks as determined by least square mean analysis (α=0.05).

Treatment	Species	ε	P_{max}	LCP	95%L_{sat}
Post-harvest	P. crista-castrensis (n=55)	0.213^{A} (0.016)	12.760^{A} (0.838)	152.937^{A} (44.661)	88.643^{A} (8.843)
	P. schreberi (n=56)	0.143^{B} (0.014)	10.952^{A} (0.848)	86.744^{A} (23.614)	192.195^{B} (21.924)
	H. splendens (n=55)	0.210^{A} (0.020)	15.880^{B} (0.942)	124.772^{A} (25.713)	264.567^{C} (27.978)
	Sphagnum (n=55)	0.296^{C} (0.026)	16.374^{B} (1.363)	88.392^{A} (17.206)	239.440^{BC} (24.346)
Unharvested	P. crista-castrensis (n=52)	0.279^{a} (0.018)	13.213^{a} (0.729)	46.436^{a} (6.124)	86.194^{a} (11.974)
	P. schreberi (n=54)	0.236^{ab} (0.023)	13.764^{a} (1.116)	104.409^{a} (30.272)	158.935^{b} (19.341)
	H. splendens (n=57)	0.221^{b} (0.014)	15.105^{a} (0.859)	69.364^{a} (15.640)	161.504^{b} (21.269)
	Sphagnum (n=59)	0.288^{a} (0.019)	13.461^{a} (0.953)	91.494^{a} (27.289)	155.763^{b} (32.374)

Table 3-2. Linear mixed model analysis results (p values) of the light curve response parameters (quantum efficiency, ε, maximum gross photosynthesis, Pmax, light compensation point, Lcp, and 95% light saturation point, 95%Lsat) for *Hylocomium splendens, Ptilium crista-castrensis, Pleurozium schreberi*, and *Sphagnum* collected in post-harvest blocks and unharvested blocks over the 2015 growing season (June – November). (n=10, except Lcp n=2-10).

Species	Light response parameter	Treatment (DF=1)	Month (DF=5)	Treatment * month (DF=5)
H. splendens	ε	0.5168	0.013	0.4582
	Pmax	0.7478	0.0054	0.1142
	Lcp	0.2550	0.0150	0.4222
	Lsat	0.0230	0.1227	0.5692
P. crista-castrensis	ε	0.0211	0.0003	0.8964
	Pmax	0.6181	0.0003	0.7536
	Lcp	0.1315	0.3075	0.8831
	Lsat	0.7406	0.0366	0.8534
P. schreberi	ε	0.0104	0.0052	0.4875
	Pmax	0.0792	<0.0001	0.1875
	Lcp	0.6575	0.0005	0.7453
	Lsat	0.3834	0.0003	0.3209
Sphagnum	ε	0.9182	0.0057	0.0356
	Pmax	0.1826	0.0045	0.0602
	Lcp	0.8201	0.0070	0.0509
	Lsat	0.0853	0.1318	0.9827

3.3.2 Drying curves

Photosynthesis rates increased slightly within the first 30 minutes of drying time for all test species, though there was not a significant difference between the rates at 0 and 30 minutes for *H. splendens*, *P. crista-castrensis*, and *P. schreberi* (Figure 3-5). The difference in % maximum photosynthesis was significant for *Sphagnum* within the first 30 minutes ($p=0.0001$; Figure 3-5). The optimal water content, ranging from 4-11 g g^{-1}, was significantly greater for the post-harvest samples of *H. splendens* ($p=0.0106$) but the difference was so small it is unlikely to have a great practical effect, and no differences were found among treatments for *P. crista-castrensis*, *P. schreberi*, and *Sphagnum* (Figure 3-5). Though no differences were found in the water contents themselves, the ability of shoots to retain water over time was greater for samples from the unharvested areas for most test species. After 2 hours the water contents were roughly 1.7 g g^{-1} for samples of *H. splendens* from both treatments; *P. crista-castrensis* and *P. schreberi* samples from the forested blocks took 30 minutes longer (2.5 hours instead of 2 hours) to reach water contents of roughly 1.4 g g^{-1} and 1.8 g g^{-1} (when photosynthesis ceased). *Sphagnum* had the most marked differences among the two treatments, samples from the post-harvest blocks attained 1.9 ± 0.2 g g^{-1} water content after 4 hours, while samples from unharvested blocks attained nearly identical levels after 6 hours (1.8 ± 0.3 g g^{-1}).

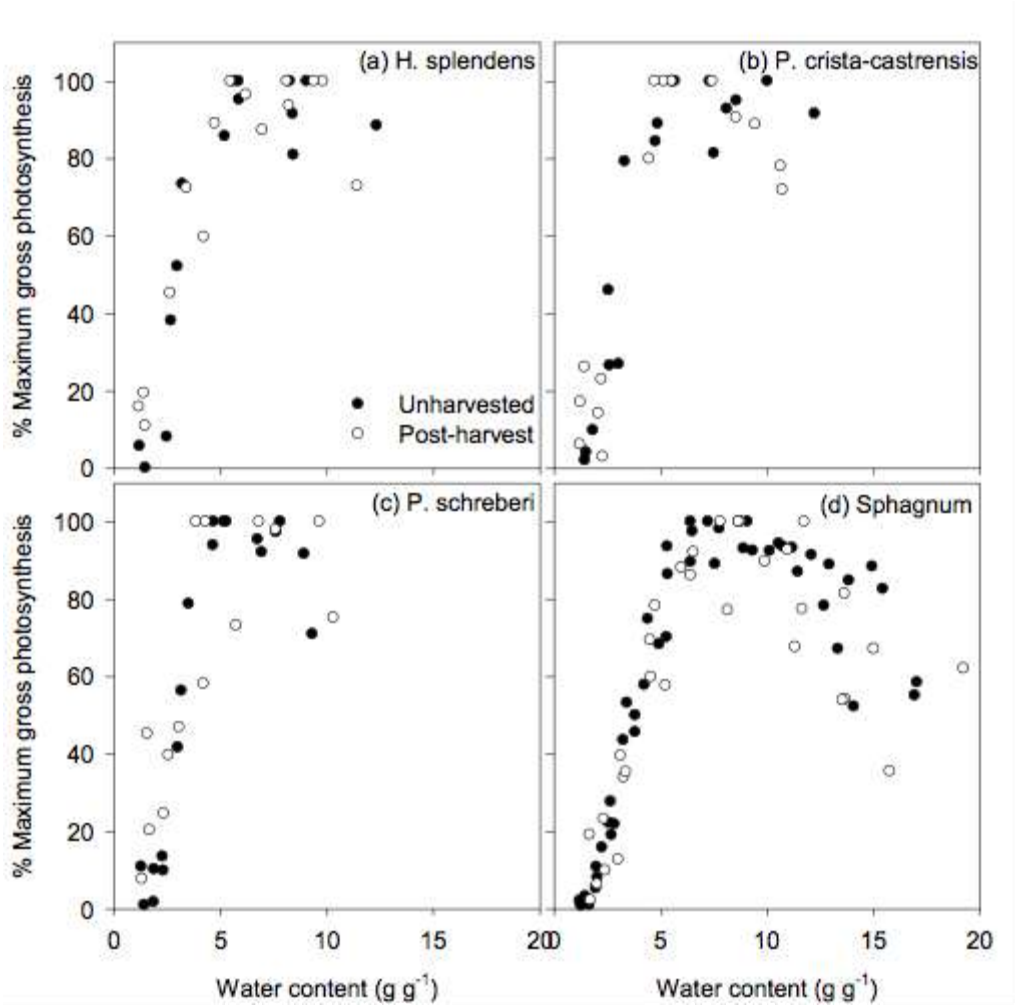

Figure 3-5. Changes in relative gross photosynthesis with decreasing water content after hydration to full turgidity in samples taken from in August 2015 from post-harvest and unharvested black spruce blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum*. (n=10)

3.3.3 Pigment analysis

Chlorophyll concentrations

Chlorophyll concentrations were almost always lower in the three feathermoss species from the post-harvest blocks over the growing season, while concentrations were often similar for *Sphagnum* between the two treatments (Figure 3-6, 3-7, & 3-8). Seasonal maximums of chlorophyll concentrations were often seen in July and August and decreased over the fall months for all species from both the post-harvest and the unharvested blocks (Figure 3-6, 3-7, & 3-8). There were no significant differences in chlorophyll concentrations among species from the post-harvest blocks over the growing season (Table 3-3). In the unharvested forest blocks, *P. schreberi* had greater concentrations of Chl *a*, Chl *b*, and total chlorophyll than all other species ($p<0.0001$) (Table 3-3).

Chl *a* concentrations for *P. crista-castrensis* ranged from 104-318 µg g^{-1} in the post-harvest blocks and 149-258 µg g^{-1} in the forested areas, *P. schreberi* ranged from 132-271 µg g^{-1} in the post-harvest blocks and 272-479 µg g^{-1} in the unharvest forested areas, *H. splendens* ranged from 92-275 µg g^{-1} in the post-harvest blocks and 127-259 µg g^{-1} in the forested areas, and *Sphagnum* ranged from 121-350 µg g^{-1} in the post-harvest blocks and 116-335 µg g^{-1} in the forest areas (Figure 3-6). Chl *a* concentrations were significantly greater in *P. schreberi* from the unharvested forest areas over all months (except July), and for *H. splendens* in June and October ($p<0.05$) (Figure 3-6). *P. crista-castrensis* and *Sphagnum* samples had no significant differences during any month between the treatments for Chl *a* concentrations (Figure 3-6).

Chl *b* concentrations for *P. crista-castrensis* ranged from 66-164 µg g^{-1} in the post-harvest blocks and 97-158 µg g^{-1} in the unharvested forest areas, *P. schreberi* ranged

from 75-130 µg g^{-1} in the post-harvest blocks and 174-277 µg g^{-1} in the forest areas, *H. splendens* ranged from 61-145 µg g^{-1} in the post-harvest blocks and 92-171 µg g^{-1} in the forest areas, and *Sphagnum* ranged from 65-202 µg g^{-1} in the post-harvest blocks and 64-212 µg g^{-1} in the forest areas (Figure 3-7). Chl *b* concentrations were significantly higher in *P. schreberi* samples from the forest sites compared to the post-harvest blocks for the entire growing season, and the same relationship was found between the treatments for *P. crista-castrensis* samples in June, and *H. splendens* samples in June, August, September, and October ($p<0.05$) (Figure 3-7).

Total chlorophyll concentrations for *P. crista-castrensis* ranged from 171-482 µg g^{-1} in the post-harvest blocks and 246-417 µg g^{-1} in the forest areas, *P. schreberi* ranged from 212-401 µg g^{-1} in the post-harvest blocks and 446-757 µg g^{-1} in the forest areas, *H. splendens* ranged from 253-421 µg g^{-1} in the post-harvest blocks and 219-430 µg g^{-1} in the forest areas, and *Sphagnum* ranged from 188-552 µg g^{-1} in the post-harvest blocks and 180-547 µg g^{-1} in the forest areas(Figure 3-8). Monthly means were significantly higher for *P. schreberi* from the forested blocks compared to the post-harvest blocks during all months (Figure 3-8), and for *H. splendens* in June and October ($p<0.05$) (Figure 3-8).

Treatment effects were significant for Chl *a*, Chl *b* and total chlorophyll concentrations for *P. schreberi* and *H. splendens* (Table 3-4). The effect of sampling month was significant for all three chlorophyll concentrations in all species (Table 3-4). There was a significant interaction between treatment and sampling month for Chl *a* concentrations in *P. crista-castrensis*, *P. schreberi*, and *H. splendens* (Table 3-4). The interaction between treatment and sampling month was significant for Chl b concentrations for all species except *P. crista-castrensis* (Table 3-4). A significant

interaction was present for total chlorophyll concentrations between sampling month and treatment for *P. schreberi* and *H. splendens* (Table 3-4).

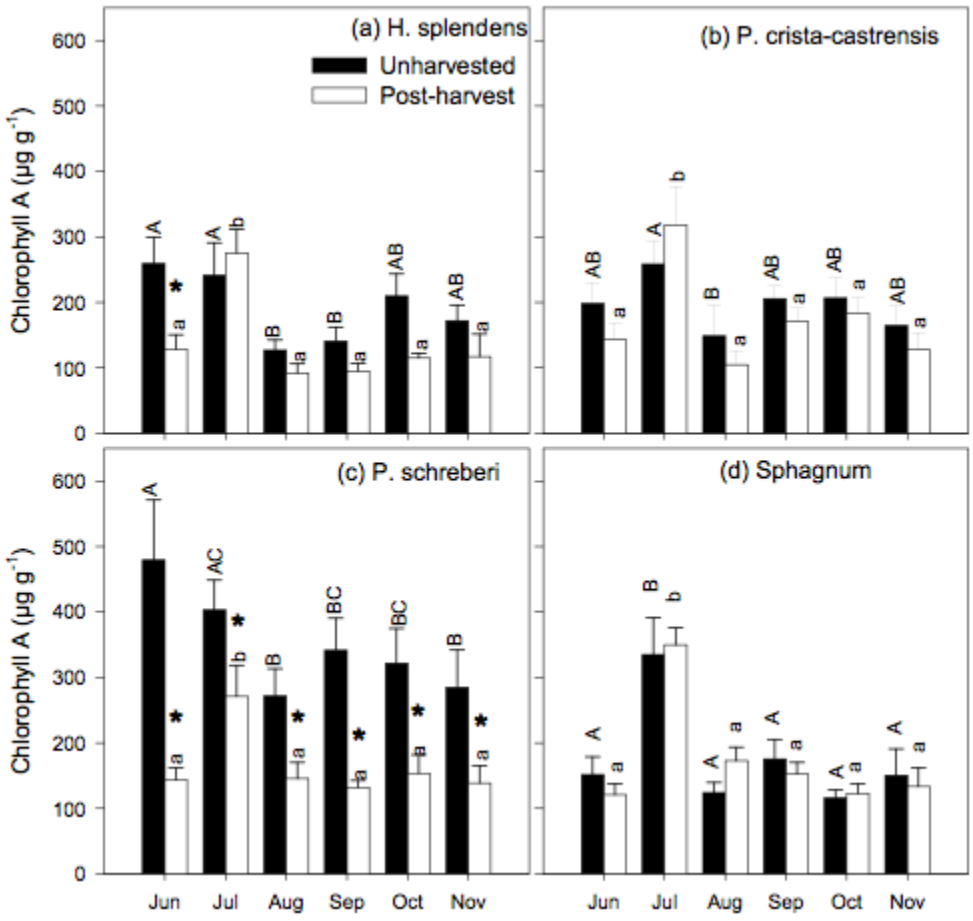

Figure 3-6. Chlorophyll *a* concentrations (Chl *a*, µg Chl *a* / g dry moss, with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the forested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

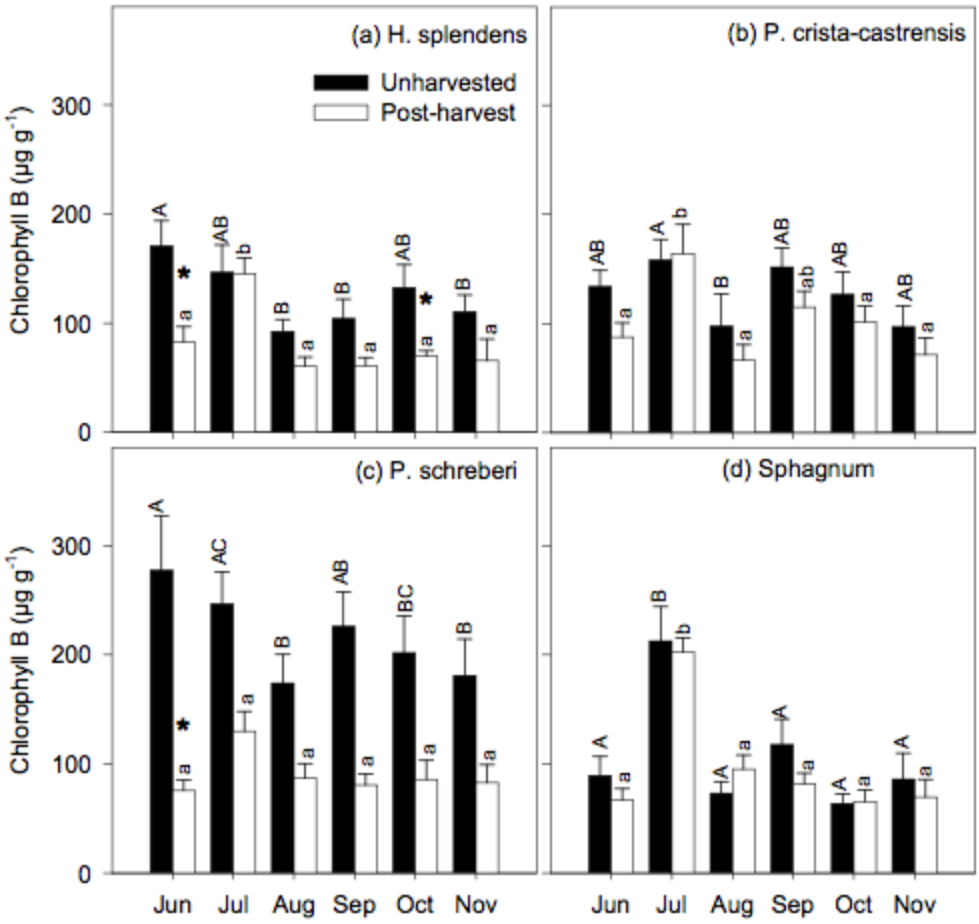

Figure 3-7. Chlorophyll *b* concentrations (Chl *b*, µg Chl *b* / g dry moss, with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the forested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

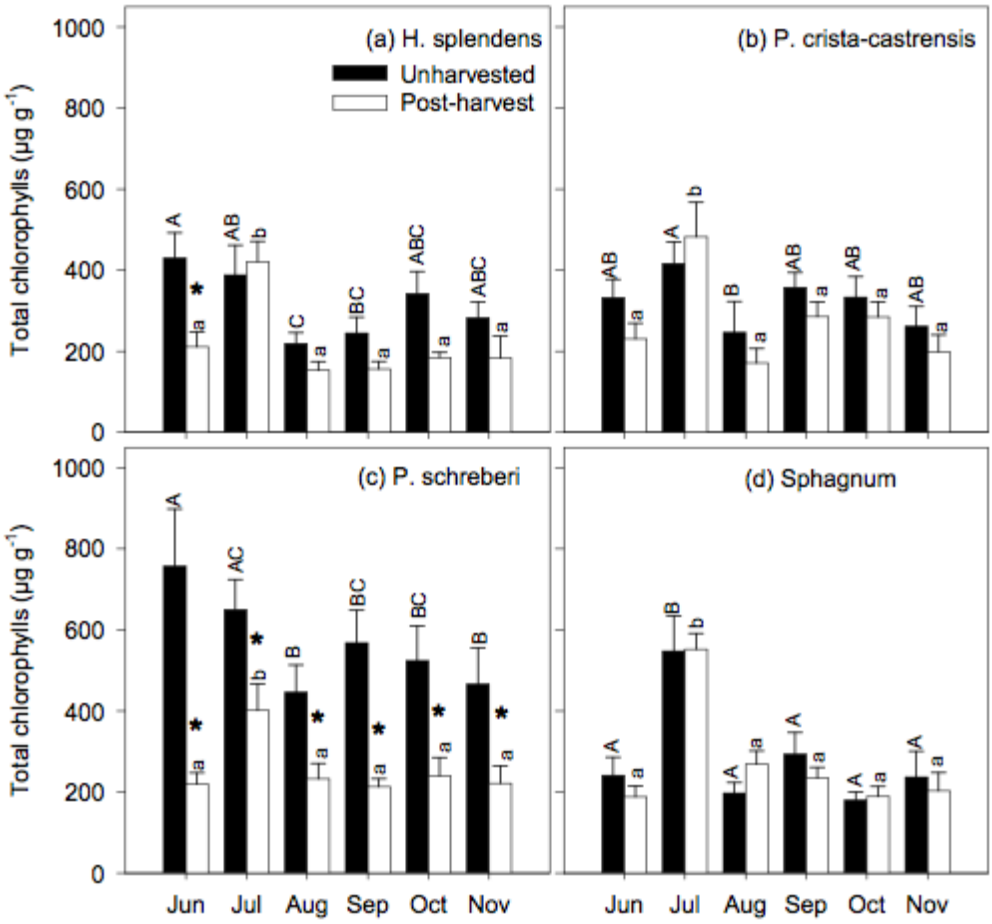

Figure 3-8. Total chlorophyll (Chl *a* + Chl *b*) concentrations (µg Chl / g dry moss, with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the forested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Carotenoids

The concentration of carotenoids in all species peaked and attained minimums over the same months as concentrations of chlorophylls (Figure 3-8). Carotenoid concentrations were often lower in samples from the post-harvest blocks, though concentrations in the samples of *Sphagnum* remained quite similar between the treatments (Figure 3-9). Carotenoid concentrations for *P. crista-castrensis* ranged from 44-126 µg g^{-1} in the post-harvest blocks and 52-85 µg g^{-1} in the forest areas *P. schreberi* ranged from 65-144 µg g^{-1} in the post-harvest blocks and 97-149 µg g^{-1} in the forest areas, *H. splendens* ranged from 48-126 µg g^{-1} in the post-harvest blocks and 56-86 µg g^{-1} in the forest areas, and *Sphagnum* ranged from 43-91 µg g^{-1} in the post-harvest blocks and 47-96 µg g^{-1} in the forest area (Figure 3-9). For the post-harvest blocks and the forest areas, *P. schreberi* had higher carotenoid concentrations than both *Sphagnum* (post-harvest, $p=0.0011$; forest, $p<0.0001$) and *H. splendens* (post-harvest, $p=0.0351$; forest, $p<0.0001$), and in the forested areas *P. schreberi* also had greater concentrations than *P. crista-castrensis* ($p<0.0001$) (Table 3-3). The treatment effect on carotenoid concentration was only significant in *P. schreberi*, but the effect of sampling month was significant for all species (Table 3-4). Over the growing season, there was a significant interaction between treatment and sampling month in *P. crista-castrensis*, *P. schreberi*, and *H. splendens* (Table 3-4). Carotenoid concentration had a highly significant positive correlation to total chlorophyll concentration, and a highly significant negative relationship to the ratio of chlorophyll: carotenoids and Chl *a:b* ($p<0.0001$). A positive relationship was also found with concentrations of Chl *a* ($p=0.0001$) and Chl *b* ($p=0.0013$).

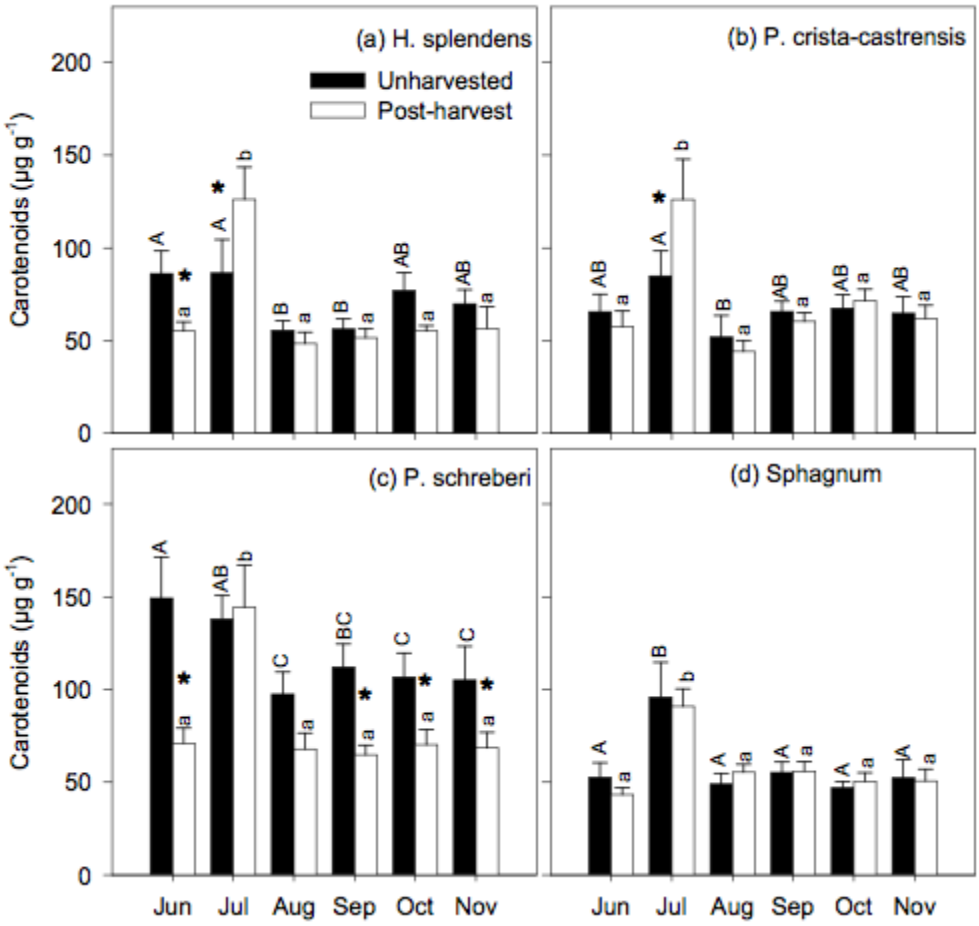

Figure 3-9. Carotenoid concentrations (µg carotenoid / g dry moss, with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the unharvested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Ratio of chlorophyll a:b

The ratio of Chl *a:b* generally ranged from 1.3-2.0 for all species over the growing season (Figure 3-10). Ratios were always higher in the samples from the post-harvest blocks, though this relationship in a given month was only significant in the month of June for *Sphagnum* samples (Figure 3-10). Seasonal maximums of the Chl *a:b* ratio were seen in the post-harvest blocks in June and July (Figure 3-10). Samples from the unharvested forest areas attained maximum ratios in June for *P. schreberi*, July for *H. splendens*, October for *Sphagnum*, and November for *P. crista-castrensis* (Figure 3-10). Within samples from the unharvested forest areas the only significant difference among species was a larger ratio for *Sphagnum* than for *H. splendens* ($p=0.0388$) (Table 3-3). In the samples from the post-harvest blocks the Chl *a:b* ratio was greater in *Sphagnum* samples than all other species ($p<0.05$) (Table 3-3). A treatment effect was found significant only in the *P. schreberi*, but sampling month had significant effect on all species except for *Sphagnum* (Table 3-4). The interaction between treatment and sampling month was significant for *P. crista-castrensis* (Table 3-4). Correlation analysis found significantly positive relationships between Chl *a:b* and Chl *a*, Chl *b*, and chlorophyll: carotenoid ratio, while there was negative correlation between total chlorophyll and carotenoid concentrations ($p<0.0001$).

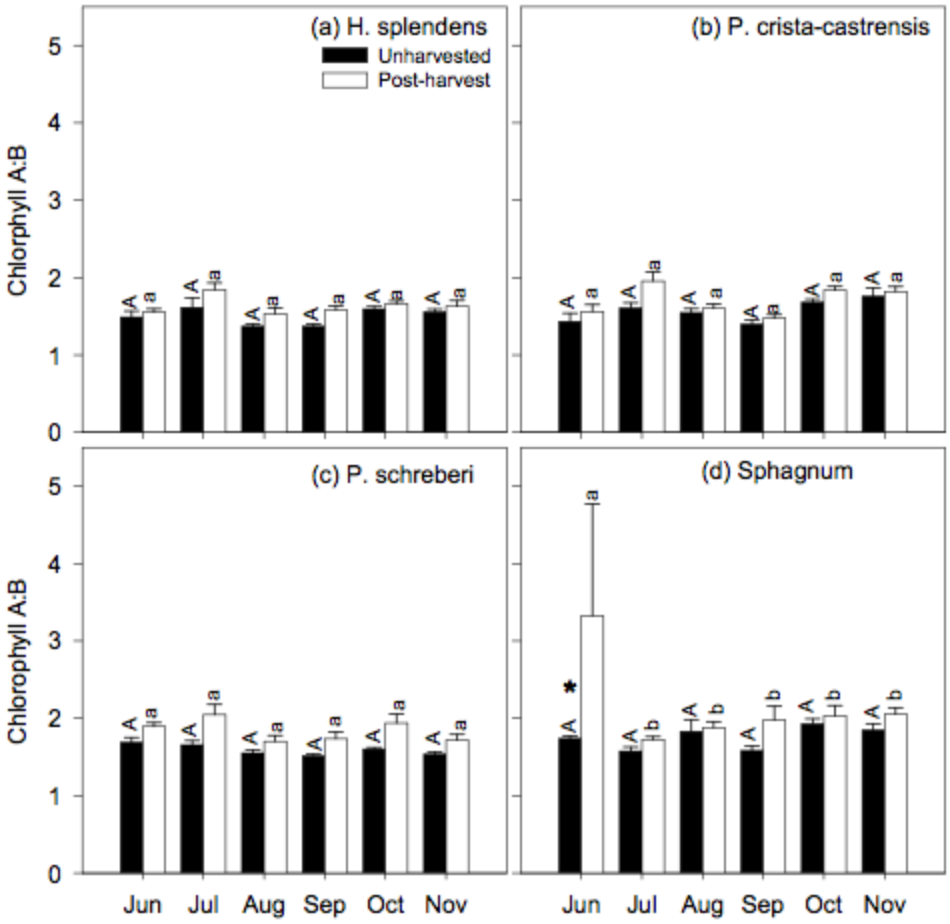

Figure 3-10. Chlorophyll *a:b* ratio (g Chl *a*/ g Chl *b*, with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the forested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Ratio of total chlorophyll to total carotenoids

The ratio of total chlorophylls: carotenoids ranged from 2.8-6 over the season for all species, and were lower in the samples from the post-harvest blocks (Figure 3-11). Higher ratios were found in the earlier growing season, and minimums in the later fall (Figure 3-11). In the unharvested forest areas, the ratios were significantly lower for *H. splendens* than for *P. crista-castrensis* ($p=0.0083$) and *P. schreberi* ($p=0.0359$) (Table 3-3). For samples from the post-harvest blocks, *Sphagnum* had a greater value for the ratio than all three feathermosses (*H. splendens*, $p<0.0001$; *P. crista-castrensis*, $p=0.0002$; *P. schreberi*, $p<0.0001$), and *P. crista-castrensis* had a greater ratio than both *P. schreberi* ($p=0.0002$) and *H. splendens* (Table 3-3). The effect of treatment was significant for *P. crista-castrensis*, *P. schreberi*, and *H. splendens*, leading to decreases in the ratio in the post-harvest blocks (Table 3-4). The effect of sampling month was highly significant for all species except *P. schreberi*, and a significant interaction between treatment and sampling month was detected in *Sphagnum* (Table 3-4).

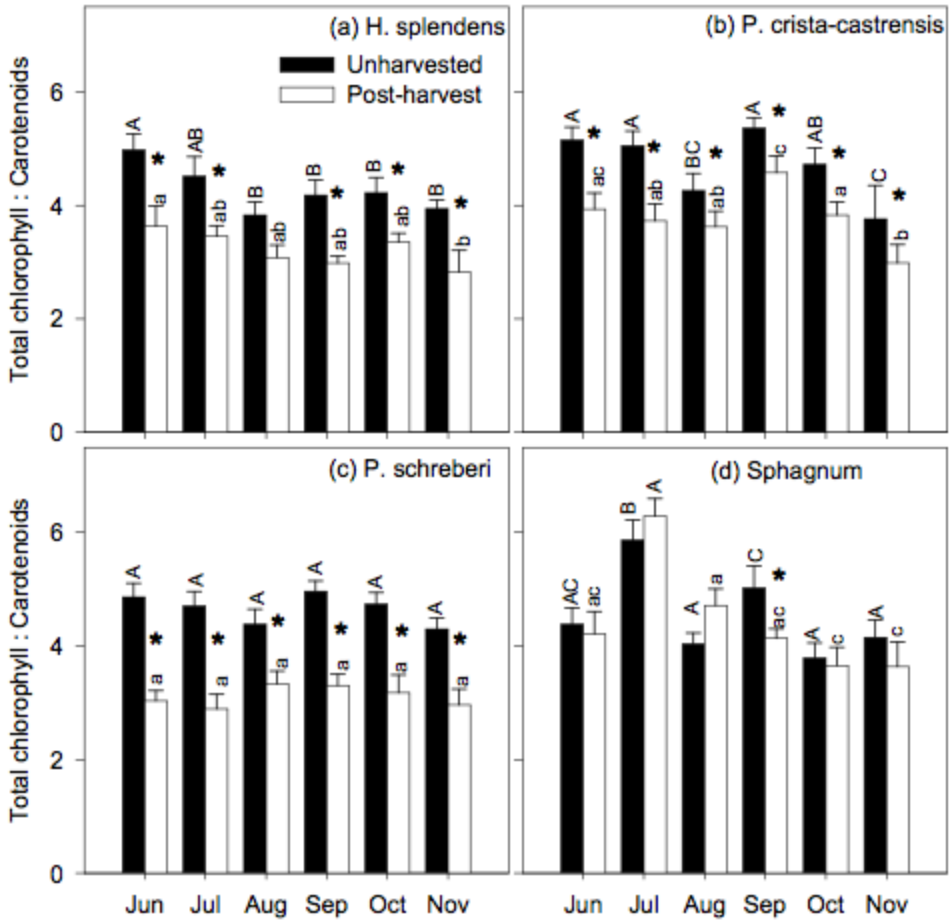

Figure 3-11. Chlorophyll :carotenoid concentration ratio (with standard error) from June-November 2015. Values are averages of 30 samples from the post-harvest and the unharvested blocks for A) *Hylocomium splendens*, B) *Ptilium crista-castrensis*, C) *Pleurozium schreberi*, and D) *Sphagnum* spp. (n=30). Different uppercase letters denote significant differences between months in the unharvested treatment, and differences in lowercase letter denote significant differences between months in the post-harvest blocks. A * denotes a treatment effect in a given month. ($p<0.05$).

Table 3-3. Seasonal means (with standard error in parentheses) of photosynthetic pigment concentrations and ratios for *P. crista-castrensis*, *P. schreberi*, *H. splendens*, and *Sphagnum* collected in post-harvest and unharvested forest blocks over the 2015 growing season (June – November). Uppercase letters represent differences in values among species within the post-harvest blocks; lowercase letters denote differences among species within the unharvested blocks as determined by least square mean analysis (α=0.05). (n=180).

Treatment	Species	Chl a ($\mu g\ g^{-1}$)	Chl b ($\mu g\ g^{-1}$)	Carotenoids ($\mu g\ g^{-1}$)	Total Chl ($\mu g\ g^{-1}$)	Chl a : b	Chl : Car
Post-harvest	P. crista-castrensis	175.464^{A} (15.507)	101.451^{A} (7.904)	70.389^{AB} (5.399)	276.915^{A} (23.203)	1.708^{A} (0.037)	3.793^{A} (0.125)
	P. schreberi	164.670^{A} (12.963)	90.566^{A} (6.218)	81.453^{A} (6.001)	255.237^{A} (18.933)	1.836^{A} (0.040)	3.118^{B} (0.099)
	H. splendens	138.920^{A} (12.806)	81.621^{A} (6.413)	66.269^{B} (5.372)	220.541^{A} (19.090)	1.635^{A} (0.030)	3.222^{B} (0.106)
	Sphagnum	177.151^{A} (13.535)	97.818^{A} (8.060)	58.132^{B} (3.111)	274.969^{A} (21.494)	2.146^{B} (0.227)	4.458^{C} (0.175)
Unharvested	P. crista-castrensis	199.675^{a} (13.872)	129.170^{a} (8.630)	67.349^{a} (4.052)	328.845^{a} (22.201)	1.576^{ab} (0.034)	4.754^{a} (0.120)
	P. schreberi	353.111^{b} (25.243)	218.810^{b} (14.671)	118.731^{b} (6.622)	571.921^{b} (39.753)	1.595^{ab} (0.020)	4.660^{a} (0.096)
	H. splendens	191.945^{a} (14.542)	126.511^{a} (8.434)	71.912^{a} (4.517)	318.457^{a} (22.701)	1.497^{a} (0.029)	4.282^{b} (0.115)
	Sphagnum	175.869^{a} (16.337)	107.426^{a} (10.450)	58.758^{a} (4.460)	283.295^{a} (26.640)	1.747^{b} (0.037)	4.548^{ab} (0.151)

Table 3-4. Linear mixed model analysis results (p values) of the photosynthetic pigment concentrations and ratios (chlorophyll a and b, Chl *a* and Chl *b*, total chlorophyll, carotenoids, the ratio of chlorophyll a:b, Chl a:b, and the ratio of total chlorophylls to carotenoids, chl:carotenoids) for *Hylocomium splendens*, *Ptilium crista-castrensis*, *Pleurozium schreberi*, and *Sphagnum* collected in post-harvest blocks and unharvested blocks over the 2015 growing season (June –November). (n=360).

Species	Pigment	Treatment (DF=1)	Month (DF=5)	Treatment*month (DF=5)
H. splendens	Chl *a*	0.0379	<0.0001	<0.0001
	Chl *b*	0.0285	<0.0001	0.0001
	Carotenoids	0.2473	<0.0001	<0.0001
	Total chlorophyll	0.0303	<0.0001	<0.0001
	Chl a:b	0.1815	<0.0001	0.3011
	Chl:carotenoids	0.0103	<0.0001	0.3171
P. crista-castrensis	Chl *a*	0.5365	<0.0001	0.0276
	Chl *b*	0.262	<0.0001	0.2251
	Carotenoids	0.6458	<0.0001	0.0002
	Total chlorophyll	0.4094	<0.0001	0.0646
	Chl a:b	0.0994	<0.0001	0.0037
	Chl:carotenoids	0.0285	<0.0001	0.0513
P. schreberi	Chl *a*	0.0043	<0.0001	0.0004
	Chl *b*	0.0054	<0.0001	0.0007
	Carotenoids	0.0117	<0.0001	0.0003
	Total chlorophyll	0.0046	<0.0001	0.0005
	Chl a:b	0.003	<0.0001	0.2857
	Chl:carotenoids	0.0006	0.0583	0.0653
Sphagnum	Chl *a*	0.7002	<0.0001	0.1411
	Chl *b*	0.4247	<0.0001	0.0219
	Carotenoids	0.6295	<0.0001	0.4447

	Total chlorophyll	0.5701	<0.0001	0.0818
	Chl a:b	0.1101	0.3701	0.4661
	Chl:carotenoids	0.6665	<0.0001	0.0024

3.4 Discussion

3.4.1 Environmental conditions

Over the 2015 growing season the ground cover of the post-harvest areas received greater levels of incoming radiation and had higher air temperatures (Figure 2-2), as was expected based on other studies on the effects of clear-cutting (Arsenault *et al.* 2012; Palviainen *et al.* 2005). Daytime PAR values were much higher in the post-harvest areas than in the unharvested blocks because of the low density of regenerated young stands 12 years after the harvest occurred (Figure 2-2). The daytime temperature of the post-harvest blocks was rarely outside a reasonable range for boreal moss species' fitness, while temperature in the unharvested blocks was slightly more often within the optimal range of 15-25 ˚C over the growing season (Figure 2-2; Furness and Grime, 1982). Given the temperature range seen over the growing season, growth inhibition due to high temperatures was an unlikely factor in the differences seen for photosynthetic or pigment metrics between the post-harvest and unharvested stands. However, the decrease in photosynthetic and pigment values in November (Figure 3-8 & 3-9) could be attributed to the negative physiological impacts on moss shoots of repeated freeze-thaw cycles (Kennedy, 1993) due to diurnal temperatures variation, as there were 11 frost events in November (data not shown).

3.4.2 Light response parameters

Rates of ε were within the range of those published by others, and the greater rates in the forested blocks suggests that the studied moss species are better suited to grow in these low light environment (Kangas *et al.* 2014; Núñez-Olivera *et al.* 2005). Bergeron *et*

al. (2009) found higher rates of ε for *Sphagnum* than for feathermoss species, which was also found in this study (Table 3-1 & Figure 3-1). The real-world effects of changes in this photosynthetic parameter for mosses have been questioned, as species common to the boreal are considered shade plants and the number of hours where irradiance levels fall within the range where ε is a constraint on photosynthesis could be quite minimal (Davey and Rothery, 1996).

The values for light compensation point (Lcp) found in this study are similar in range to the levels found by Sonesson *et al.* (1992) and Kubásek *et al.* (2014) for mosses (Figure 3-3), and are in line with the classification of these mosses as shade plants (Marschall and Proctor, 2004). A study conducted by Gaberščik and Martinčič (1987) found few seasonal changes in the Lcp values over the growing season; though in our study the few seasonal changes apparent upon analysis were likely due to the extreme rates of variation within species which could be included based on net CO_2 exchange rates which would potentially hit the CO_2 compensation point (Figure 3-3).

95% light saturation ($95\%L_{Sat}$) values found were in agreement with our study hypothesis, with greater light levels needed to reach P_{max} rates in samples from the post-harvest areas (i.e. high light environment) (Figure 3-4), and the light values found were within the range reported by others for mosses (Harley *et al.* 1989; Bergeron *et al.* 2009; Jägerbrand *et al.* 2012; McCall and Martin, 1991; Marschall and Proctor, 2004). $95\%L_{Sat}$ levels of all species suggest they were much more shade-adapted than bryophyte species more commonly found in peatlands or open areas (Clymo and Hayward, 1982; Rice *et al.* 2008). The samples from the forested blocks saturated at levels close to monthly mean light intensities as expected, and may have been light limited near the end of the growing

season (Bergeron *et al.* 2009; Bisbee *et al.* 2001). Measured PAR values for the post-harvest blocks were much greater than saturating light levels calculated for samples in these areas (Figure 2-2 & 3-4), a response observed before by Marschall and Proctor (2004) who found saturating light levels which equaled only 50% of the regular daytime irradiance for moss species in some high light environments. Given this overabundance of light in the post-harvest blocks as well as temperatures which remained mostly within an acceptable range for the moss species (Figure 2-2), moisture is left as the most likely limiting productivity factor in the post-harvest samples (Bergeron *et al.* 2009; Furness and Grime, 1982).

The rates of maximum photosynthesis (P_{max}) found in this study were similar or slightly greater than those found by others (Figure 3-2), and the variability among and within species is not uncommon (Harley *et al.* 1989; Wang *et al.* 2014; Liu *et al.* 2001; Rice *et al.* 2008). However, rates found over most months were contrary to our original hypothesis that P_{max} would always be greater in the samples from the post-harvest blocks, suggesting that the mosses from the post-harvest blocks were not able to fully capitalize on their high light environment, again likely a response to moisture stress (Bergeron *et al.* 2009) (Table 3-1 & Figure 3-2). The narrow growth form and compact foliage of black spruce is such that ground vegetation in the forested blocks was likely subjected to frequent sun-flecks (bursts of high incoming radiation), and strong seasonal changes in irradiance at the forest floor due to changing angles of incoming solar radiation (Swanson and Flanagan, 2001; Kubásek *et al.* 2014; Pearcy, 1990; Davey and Rothery, 1996). Some species of mosses can utilize these intervals of high light intensity very efficiently, and have been observed to have greater growth rates in changing light environments than in

constant light regimes, whether high or low intensity (Rincon and Grime, 1989). Potentially, this could have also been a reason for the lack of a more consistent difference in photosynthetic parameters of mosses between the unharvested and the post-harvest blocks (Table 3-2).

P_{max} peaked over the mid-summer months and decreased during the fall, following the trend of air temperatures and number of daylight hours (Figure 2-2 & 3-2), as was expected based on a review of the available literature (Jägerbrand *et al.* 2012; Gaberščik and Martinčič, 1987). Jägerbrand *et al.* (2012) found the greatest rates of P_{max} in August when mosses stopped growing apically and shifted their resource allocation from physical growth to photosynthetic pigment production, potentially depicted in the present study based on increasing pigments in September (Figure 3-2 & 3-8).

Results in the present study resembled those of Goulden and Crill (1997) who found greater P_{max} rates for *Sphagnum* than for feathermosses within the post-harvest blocks (Table 3-1, Figure 3-2). The large water holding capacity of *Sphagnum* shoots (Figure 3-5) results in wetter soils under *Sphagnum* mats than under feathermosses, and the stems themselves retain more moisture after rainfall events (Bisbee *et al.* 2001; Wang *et al.* 2014). This theory suggests that *Sphagnum* shoots could have had a greater total number of photosynthetically active hours over the season, and is supported by the higher Chl *a:b* ratio found for *Sphagnum* shoots from the post-harvest areas than the feathermoss shoots (Figure 3-10) suggesting that *Sphagnum* shoots were photosynthetically active during more periods of high light than feathermosses (Lichtenthaler *et al.* 2013). Additionally, due to the wetter soil conditions under *Sphagnum* mats they may have endured less moisture stress, enabling a greater allocation of resources to photosynthetic

apparatus and decreasing any potential treatment effects (Table 3-2) (Rice, 1995; Rice *et al.* 2008). It is possible also that the interspecies differences in P_{max} were driven by differences in the relative measure of leaf area to mass between species, as there was a significant difference in this physiological characteristic (Table 2-5), though studies differ in the findings of whether this can be negatively correlated to photosynthetic parameters or not (Rice *et al.* 2008; Waite and Sack, 2010).

3.4.3 Photosynthetic pigments

The decreased concentrations of chlorophylls observed in mosses sampled from the post-harvest blocks (Figure 3-8) are in line with the results of other studies where habitat irradiance was negatively correlated with total chlorophyll concentration on a dry mass basis (Marschall and Proctor, 2004; Lichthenthaler *et al.* 2013). Often, mosses in low light environments (such as in the forested blocks) increase the proportion of energy expended on photosynthetic pigments relative to structural components in order to maximize potential light interception, and likely adding to this treatment effect some mosses have been found to decrease chlorophyll concentrations at apical tips in high light environments such as in the post-harvest areas as a means of photo-protection (Czeczuga 1987; Rincón 1993; Kershaw and Weber, 1986; López and Carballeira, 1989; Tobias and Niinemets, 2010). In agreement with the chlorophyll content findings and study hypothesis, the ratio of Chl *a:b* was greater in the post-harvest areas because of more available light (Figure 2-2 & 3-10); increasing the relative concentrations of Chl *b* is a response of plants to low light conditions and aids to maximize the ability of chloroplasts to best harvest the limited amounts of light (Dale and Causton, 1992;). The significant

treatment effects observed across measured pigments for *P. schreberi* (Table 3-4) has also been previously recorded by Tobias and Niinemets (2010), who noted pigment concentrations were up to 400% greater in *P. schreberi* samples from heavily shaded areas compared to those from high light environments.

Although carotenoid differences were much smaller than those of total chlorophylls (Figure 3-8 & 3-9), the measured carotenoid concentrations are similar in range to values published by others (Czeczuga, 1987; Núñez-Olivera *et al.* 2005) (Table 3-3). The unexpected decrease in concentrations in the post-harvest areas suggests that in the current scenario the specific types of carotenoids present may be shifted towards those which aid light absorption as opposed to those involved in photo-protection (Boston *et al.* 1991; Lappalaien *et al.* 2008; Núñez-Olivera *et al.* 2005; Rice *et al* .2008).

The total chlorophyll concentrations and the ratio of Chl *a:b* (Figure 3-8 & 3-10) were within the range reported for mosses from shaded woodland areas (McCall and Martin, 1991; Marschall and Proctor, 2004; Lichtenthaler *et al.* 2013; Núñez-Olivera *et al.* 2005; Rincón, 1993; Martin, 1980; Hoddinott and Bain, 1979; Tobias and Niinemets, 2010). Typical irradiance values at the forest floor of black spruce dominated boreal forest stands range from 15-30% of that which hits the tree canopies, a range found also in the present study and higher than levels reported for other tree stands (Figure 2-2), and these greater light levels could have led to the discrepancies in values found (Table 3-3 & Figure 3-10) (Bergeron *et al.* 2009; Gower *et al.* 2001; Swanson and Flanagan, 2001). Even in the post-harvest areas, shading of vascular plants and shrubs can create a more variable light environment for mosses than suggested by logged PAR (Figure 2-2) (Bisbee *et al.* 2001). Low Chl *a:b* ratios and pigment contents could also have been found

due to greater self-shading within the moss canopies, decreasing light available (and photosynthetic capacity) for lower shoot segments and increasing senescence at depth (Gerdol *et al.* 1994).

The ratio of chlorophyll : carotenoids was as expected lower in the post-harvest sites (Figure 3-11), due to the greater response of chlorophyll content to increasing irradiance compared to changes in carotenoids (Marschall and Proctor, 2004; Tobias and Niinemets, 2010; Lappalainen *et al.* 2008). Values of the ratio were within the ranges published by others (Marschall and Proctor, 2004; Gerdol *et al.* 1994; Rice *et al.* 2008), though the samples from the post-harvest areas were on the lower end of those previously reported (Table 3-3).

The current study didn't detect a strong seasonal trend in total chlorophyll concentrations as has been found by some studies, though some species exhibited a drop in concentrations later in the growing season (Figure 3-8) (Kershaw and Weber, 1986; Lappalainen *et al.* 2008), but the lack of a seasonal trend was also found by Davey and Rothery (1996). The hospitable microclimate in the post-harvest areas due to higher rainfall and a lower average temperature in the month of June (Figure 2-2) may have allowed shoots to allocate a greater proportion of energy to photosynthetic pigments as opposed to structural components, as viewed by the spike in concentrations for the samples collected in July (Figure 3-8) (McCall and Martin, 1991; Rice 1995). The lack of a strong seasonal trend in the ratio of Chl *a:b* measured in this study (Figure 3-10) has also been observed in the feather moss *Brachythecium rutabulum* (Kershaw and Webber, 1986), and the same was found for the ratio of chlorophyll: carotenoids by Marschall and

Proctor (2004), and both may be due to the correlations between pigments found in the present (Johnson *et al.* 1993).

3.4.4 Impacts of clear-cutting

The poikilohydric nature of mosses means that photosynthetic characteristics are often driven more by moisture than light regimes (Ueno *et al.* 2006, Williams and Flanagan, 1998), and one way by which mosses can prolong periods of photosynthesis (i.e. defer water loss) when growing in more challenging areas of high light or temperature and low moisture is to grow in denser mats which more effectively retain water (Table 2-5) (Bergamini *et al.* 2001; Lindo and Gonzalez, 2010). However, this increase in shoot density can decrease the ability of light to reach shoot segments at depth, leading to senescence of shoots closer to the surface and decreases in photosynthetic capacity per unit of stem length (Niinemets and Tobias, 2014). If the increased temperatures and light levels in the post-harvest areas (Figure 2-2) altered the shading properties and light attenuation within the moss canopy due to changes in moss mat density as suggested by the shoot density counts (Table 2-5), the amount of photosynthetically active tissues within the top 2 cm of shoots used for photosynthetic analysis could have been quite different between the treatments, even though effort was taken to use only green stems (Niinemets and Tobias 2014; Tobias and Niinemets, 2010). Changing pigment concentrations or photosynthetic activity at depth have been found for both *Sphagnum* and feathermosses, with a curvilinear change in pigment with depth but often a near linear decrease in photosynthetic ability (Niinemets and Tobias, 2014; Schmidt-Stohn 1977; Gerdol *et al.* 1994; Sonesson *et al.* 1992). Additionally, the

degradation of chlorophyll in shoots from years past could impact the upper segments tested in this study, and potentially this is another factor leading to the low pigment concentrations measured in this study generally (Gerdol *et al.* 1994). Overall, the impacts of clear-cutting could be measured in many photosynthetic parameters and pigment measures consistently over the growing season, potentially altering the net ecosystem carbon exchange within these areas.

3.4.5 Species impacts

Marschall and Proctor (2004) suggested that although microclimate can impact pigment concentrations and ratios, the variation cannot be explained solely by abiotic factors. Responses to microclimate changes are often species specific, and can cover a range of potential morphological and functional traits (Hyyrläinen *et al.* 2015). For example, pigment concentrations and photosynthetic capacity could be affected by the ratio of cell walls to cell contents of shoots; a denser or larger cell wall would decrease the pigment concentration in a given species while not truly altering photosynthetic capacity, or the same effect could be due to differences in the ratio of photosynthetic leaves to non-photosynthetic stems (Marschall and Proctor, 2004; McCall and Martin, 1991). An effect such as this could have led to the lack of a treatment effect for *P. crista-castrensis* and *Sphagnum* samples over the season (Table 3-4), both species which had variability in SLA and shoot density counts (Table 2-5).

Especially in the post-harvest areas, the increase in rates of P_{max} measured in *Sphagnum* samples (Figure 3-2) may have been an example of the positive outcomes of the water retention ability (Figure 3-5); the stems were able to stay sufficiently hydrated

over time and actually increase photosynthetic output over a season (Rice *et al.* 2008). *Sphagnum* samples did seem more able to capitalize on the higher light levels present in the post-harvest areas to increase photosynthetic capacity (Figure 3-2), especially during the early summer months when moisture was abundant and not a limiting factor (Bisbee *et al*. 2001). The differences in photosynthetic rates and pigment ratios between *Sphagnum* and feathermosses could also be the results of different growth forms (Table 3-1 & 3-3). *Sphagnum* shoots are erect, which helps to minimize water loss but also increases self-shading within the moss canopy, while the feathermoss species have a prostrate growth form which extends laterally and can aid to capture most incoming radiation, but makes the stems more susceptible to drying (Benscoter and Vitt, 2007; Wang *et al.* 2016). Overall, *Sphagnum* tended to be less affected by clear-cutting in terms of pigment concentrations and photosynthetic light response parameters, having fewer statistically significant differences between test parameters (Table 3-2 & 3-4). Williams and Flanagan (1998) found that changes in photosynthetic light response parameters for *Sphagnum* species were most determined by seasonal climate conditions in the boreal region.

Our test results suggest that *P. schreberi* displayed the greatest differences in photosynthetic parameters and pigment contents between treatments (Table 3-2 & 3-4), and others have found that *P. schreberi* productivity is highly dependent on microclimate water conditions (Williams and Flanagan, 1998). Therefore, the altered microclimate conditions in the post-harvest blocks could have impacted *P. schreberi* to a greater extent, potentially due to its relatively small size and high shoot packing density (Table 2-5), leaving it more susceptible to self-shading and therefore shoot senescence at depth

(Tobias and Niinemets, 2010). The dominance of *P. schreberi* in the forested areas and strong seasonal trends measured across photosynthetic parameters and pigment concentration has been previously noted in other forests for this species (DeLucia *et al.* 2003). The differing responses of species to the harvest event could lead to a shift in species composition, with a shift towards *Sphagnum* potentially leading to paludification of the landscape as has been noted in other black spruce forests after clear-cutting (Renard *et al.* 2016).

3.5 Conclusions

Clear-cutting in boreal black spruce-moss forest can cause the harvested areas to shift to shrub-moss woodlands. The small size and simple nature of moss shoots enables them to partition resources in such an environment so that they can handle these dramatic increases in light levels over the growing season quite well, and we found that they were able to utilize beneficial conditions early in the season to bolster pigments and flux parameters. A distinct difference was seen for all feathermoss species in regards to pigment content and ratios, though this did not always translate to altered photosynthetic parameters, suggesting that moss shoots can quite adequately conserve photosynthetic abilities with altered microclimate conditions.

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Chapter 4: Conclusions

4.1 Overview of chapters

The objective of this study was to examine the longer-term responses of mosses to clear-cutting within a boreal black spruce forest in western Newfoundland. Given the broad range of forests which are clear-cut annually, and the interest in better accounting for C fluxes, the effects of harvesting on the productivity of forest floor plant life is highly important. Whole tree harvesting in an area is known to increase temperatures and incoming light levels at the ground, and these can be potentially harmful for the existing plant life (Arsenault *et al.* 2012). Mosses are potentially more sensitive to these habitat changes than other species, as they lack the suite of common water retention features of vascular plants, and are prone to drying out (Benscoter and Vitt, 2007; Marschall and Proctor, 2004; Proctor 1990).

Chapter 2 focused on the instantaneous photosynthesis rates of samples for the test species along a range of microclimate conditions which occur as a result of harvesting, and the study also tested for differences in biomass increases for shoots in both the post-harvest and unharvested forest areas. Measurements of photosynthesis and shoot growth were made over a growing season for *Sphagnum* and three feathermosses (*Hylocomium splendens*, *Ptilium crista-castrensis* and *Pleurozium schreberi*) commonly found in black spruce forests in Newfoundland, Canada. The measurements were made in open areas of post-harvest blocks (clear-cut a decade ago), along the edge of the unharvested forest blocks, and within the interior of unharvested forest areas, and consisted of both instantaneous photosynthesis readings and an analysis of light and water responses of photosynthesis. Over the entire season the environmental conditions at the ground in the

post-harvest blocks were warmer, brighter, and drier than the adjacent unharvested forest blocks. Tests confirmed that mosses could utilize the greater light levels found in the more open areas of the post-harvest test blocks, but also found that samples along the forest edge had similarly high rates of photosynthesis even when tested at significantly lower light levels. In the laboratory tests of light responses of all four test species displayed saturating light levels of under 400µmol m^{-2} s^{-1}, after which point photo-inhibition negatively affected photosynthesis rates. Biomass growth appeared to be greater for feathermosses in the forest blocks compared to the post-harvest blocks, and for all species natural shoot densities were lower in the mats collected from the unharvested blocks. The rates of instantaneous photosynthesis measured with the photosynthetic active radiation (PAR) mirroring ambient light levels were lower for samples from the forest interior for feathermosses and *Sphagnum*, and often greater for samples from the post-harvest blocks. The high instantaneous photosynthesis rates which occurred along the edges of the forest blocks, even though they were tested at much lower light intensities, indicates that this is a highly suitable growth environment for the study species. A difference was found for mat densities between treatments, with an increase across species in the post-harvest blocks, likely as a functional response to limit water loss and decrease the negative effects of the high light environment (Bergamini *et al.* 2001; Lindo and Gonzalez, 2010; Niinemets and Tobias, 2014). Overall, the low growth rates seen in the post-harvest areas suggest that the elevated photosynthesis rates found were not maintained for a sufficient length of time, presumably due to desiccation, to increase the overall productivity in the post-harvest blocks when compared to mosses along and within the unharvested forest areas.

Chapter 3 tested for the changing photosynthetic responses of test species to light intensity in samples from the post-harvest and forested tests areas. The goal was to assess potential effects of growing within the post-harvest environment on moss response to changing light levels and photosynthetic pigments contents over a growing season, and additional tests were performed to assess optimal water contents for species from both post-harvest and unharvested blocks. Tests for light responses were done by measuring CO_2 exchange rates of samples at steadily decreasing light levels, and then modelling the curves to determine biologically meaningful coefficients such as maximum photosynthesis rates, apparent quantum efficiency, light compensation points, and light saturation levels. A similar test was performed to measure the dehydration response, with photosynthesis rates measured at set time intervals as moss shoots were left to naturally dry. Photosynthetic pigments were measured monthly through spectrophotometric analysis. The light response curves created suggest that photosynthetic capacity varies strongly over the season, with peaks in productivity often seen in the middle months. Light response parameters were generally not affected by treatment for *Sphagnum*, with the exception of the quantum efficiency, which was greater in the post-harvest blocks. For feathermosses, the light saturation point was greater in the post-harvest blocks, while lower quantum efficiency values were measured in the post-harvest blocks for *P. crista-castrensis* and *P. schreberi*. Samples from post-harvest and unharvested blocks were not found to have different optimal water contents; however the ability of shoots to retain water was greater for samples from the unharvested blocks, especially in *Sphagnum* samples. Measured photosynthetic pigments were comparatively much more affected by their growth environment. Chlorophyll concentrations (Chl *a*, Chl *b*, total chlorophylls)

were greater in the post-harvest blocks in almost all species, while carotenoid concentrations were found to both increase and decrease within post-harvest blocks. The ratio of Chl a:b was generally greater in the post-harvest blocks, while the ratio of chlorophylls:carotenoids was greater in the unharvested blocks. Relatively few significant effects of clear-cutting were seen in tests for the photosynthetic responses, but many more were found for photosynthetic pigments. Pigment concentrations and ratios were highly different between samples from the post-harvest and unharvested areas, pointing to the different light regimes to which the samples were subjected. A strong treatment effect of greater pigment concentrations in unharvested forest samples was noted in all the feathermosses, presumably to aid in light interception, but *Sphagnum* samples had similar concentrations in both treatments, again signifying that they were more suited to the new environment due to their water retention abilities.

4.2 Significance and future directions

The results from both sets of experiments suggest that while clear-cutting has affected the local moss species, they can adapt by altering the partitioning of resources. The mosses appeared able to utilize windows of time where moisture was sufficient, and the periods of desiccation seem to of only marginally affected seasonal growth rates. Future studies should continue to improve on the applicability of the present results; arguably the greatest limitation to these results is that the actual water contents of the mosses in-situ were not known over extended periods of time. A comparative study on the water content of moss stems over the range of harvest conditions seasonally could help the better determine the number of photosynthetically active hours, and lend greater

context to the biomass increases found. Additionally this would enable the CO_2 flux measurements to be used for forest C budget scenarios, the water response and light response curves could be used to model CO_2 fluxes over a given period of time if accurate water contents could be found. In addition to this, it would be beneficial to continue to monitor areas such as this for longer time periods, as there is a lack of studies which assess effects of harvesting on the local species over time periods of more than 5 years.

An area for future research could be to determine the direct impact of moisture stress on the test species given a range of light conditions, negating any effects of density changes. This study was limited by natural rainfall and mat growth forms, and results are therefore limited to inference within only this area. It has been proposed that ex-situ light response curves can overestimate net photosynthesis by up to 40% for *Sphagnum* samples, due to differences in air temperature (Bergeron *et al.* 2009), in order to fully understand impacts of harvest events in-situ further studies of determining effects of water content on photosynthetic parameters other than P_{max} could be of value. Impacts of soil composition, and surrounding vascular plant communities could also yield significant results. Another interesting area of research could also follow along with that done by Kubásek *et al.* (2014) which studied the time required for bryophytes to reach their maximum photosynthetic rates at saturating light levels. Bryophytes were found to require less time than tracheophytes of the same habitats, assumedly due to their lack of stomata. Whether the changes in morphology and chemical composition in the present post-harvest scenarios altered the time needed to achieve maximal rates would aid to further understand the range of effects which environmental stressors can have.

A factor not considered in this study was whether the nutrient status of mosses in the post-harvested areas was significantly different than those which grew in the unharvested areas. Palviainen *et al.* (2005) reported a decrease in available soil nutrients in post-harvest sites, which was correlated with a decrease in annual biomass gains and nutrient concentrations in mosses for several years in post-harvest sites (Palviainen *et al.* 2005). The decreased dissolved organic carbon (DOC) content found by Bowering *et al.* (2016) in the very same regenerating stands as was studied here suggests that the nutrient status of the soils in previously clear-cut stands could have negatively impacted the fitness of mosses in these blocks. Therefore, further studies are needed to test harvest impacts on C and nitrogen content of moss tissue and whether this relates to instantaneous photosynthesis measurements or biomass gains in these areas.

The ability of mosses to exist in challenging environments is well documented, and some of the strategies which species employ to manage environmental stressors found by others, such as down-regulating photosynthesis, may have been implemented by the mosses in this study to help those which grew in the open sites of the post-harvest areas to limit dehydration damage over the summer (Hamerlynck *et al.* 2002). In some cases, mosses have been known to increase soluble sugar contents to increase osmolarity in cells and help regulate water loss, and whether the differences between treatment effects were due to chemical changes in the mosses could be an interesting area of future research (Nagao *et al.* 2005/2006).

More generally, future studies should continue to focus on long-term effects of harvesting on the local moss species. The differences noted between results found here and in other studies are likely driven by the lack of comparable time-frames used when

testing bryophytes; it has been suggested that for this species group studies on a shorter time scale (<5years) may not be as predictive as previously thought (Alatalo *et al.* 2015).

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